Energy Policy 37 (2009) 3428–3437

Contents lists available at ScienceDirect

Energy Policy

journal homepage: www.elsevier.com/locate/enpol

Algal capture of carbon dioxide; biomass generation as a tool for greenhouse gas mitigation with reference to New Zealand energy strategy and policy

Mike Packer

Cawthron Institute, MacDiarmid Institute for Advanced Materials and Nanotechnology, 98 Halifax Street, Nelson 7010, New Zealand

ARTICLE INFO

Article history:
Received 1 July 2008
Accepted 15 December 2008
Available online 20 February 2009

Keywords:
Algal biomass carbon dioxide capture
High productivity biofuel
New Zealand policy

ABSTRACT

The use of algae to capture carbon dioxide as a method for greenhouse gas mitigation is discussed. A small fraction of the sunlight energy that bathes Earth is captured by photosynthesis and drives most living systems. Life on Earth is carbon-based and the energy is used to fix atmospheric carbon dioxide into biological material (biomass), indeed fossil fuels that we consume today are a legacy of mostly algal photosynthesis. Algae can be thought of as marine and freshwater plants that have higher photosynthetic efficiencies than terrestrial plants and are more efficient capturing carbon (Box 1). They have other favourable characteristics for this purpose. In the context of New Zealand energy strategy and policy I discuss progress in growing algae and seaweeds with emphasis on their application for exhaust flue carbon recycling for possible generation of useful biomass. I also introduce schemes utilising wild oceanic algae for carbon dioxide sequestration and the merits and possible adverse effects of using this approach. This paper is designed as an approachable review of the science and technology for policy makers and a summary of the New Zealand policy environment for those wishing to deploy biological carbon sequestration.

1. Introduction

The Earth is continuously bombarded by energy from the Sun. An average of 1.2×10^{17} J/s (watts) of sunlight equates to 236 W for every square metre reaching the Earth's surface (Ramaswamy et al., 2001; Trenberth, 2001; Sension, 2007). Of this, most is absorbed directly and drives the ocean currents and our weather (eventually to be released as long-wave radiation) but a fraction (0.1–0.5%) of this is captured to enter biological systems via photosynthesis (Rabinowitch, 1961; Whitaker and Likens, 1975).

Through a couple of billion years of evolution nature has devised ways to capture the energy of the light that does fall on plants. Engel et al. (2007) show that energy captured by the photosynthetic light harvesting complex (LHC) is ~95% efficient because the structure allows quantum coherence for energy transfer between the chromophores contained within. Understanding such processes offers a great deal for bio-inspired technological advances, such as for improving the design of photovoltaic devices. Subsequent downstream steps of photosynthesis are less efficient (discussed later) but light energy captured in this way drives most living systems. The energy is used to capture atmospheric carbon dioxide (CO_2) fixing it into the biomass of life on Earth.

Presently most carbon capture and sequestration (CCS) discussions are about geological storage of CO_2. Whilst the oil and gas industry has successfully injected CO_2 into reservoirs, to date this has mainly been for increased yield of fossil hydrocarbon reserves and not for long-term storage. Even if this is proven safe, the biggest difficulty with this approach is the added cost of separation of the CO_2 from the emission streams. This paper will instead consider the use of algal systems for biological capture of CO_2 for generation of useful biomass and discuss the policy and regulatory environment in New Zealand concerning this. Carbon capture for biofuels is mitigating only in that it reduces new fossil reserves being released, by recycling carbon from the atmosphere. However, the carbon captured by algae can also be diverted to true sequestration pathways and these will also be introduced (Box 1).

2. Summary to date

This section summarises the work to date in the field of algal cultivation for CO_2 fixation and/or its conversion to biofuels. The idea is not new, having been suggested as early as 1955 (Meier, 1955) and the earliest internal combustion engines were run on biologically derived molecules (plant oils for diesel engines and bioethanol for spark-ignition engines) until plentiful petroleum-derived fuels took over due to their lower price. There are many reports on the potential and bio-economics of algal biomass to generate fuels and most of these are based on the premise that

E-mail address: mike.packer@cawthron.org.nz

0301-4215/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.enpol.2008.12.025

Box 1–Some Definitions.

Algae include seaweeds and microalgae. Many are eukaryotic organisms (those with cells displaying a high degree of internal organization, including a membrane-bound nucleus containing the genetic material and several other internal parts, organelles, that are also surrounded by membranes) but the term is often used to also include cyanobacteria (blue-green algae), which are prokaryotic (those cells that lack a distinct nucleus). There are also many species of photosynthetic bacteria such as purple-sulphur bacteria and green-sulphur bacteria but these are not referred to in the context of this paper. Algae can be either freshwater or marine, some grow optimally at intermediate saline levels and some in hypersaline conditions.

Seaweeds are macroscopic multicellular algae that have defined tissues containing specialised cells. The degree of specialisation or differentiation of cell types is much less than for terrestrial vascular plants. Again these can be marine or freshwater.

Microalgae. As the name suggests these are microscopic algae. Many are unicellular and can be motile or non-motile depending on the presence of flagella. Where multi-cellular conglomerations exist very little specialisation of cell types occurs, distinguishing them from seaweeds. There are a huge range of different types of microalgae including (non-exclusively) dinoflagellates, the green algae (Chlorophyceae), the golden algae (Chryosophyceae) and diatoms (Bacillariophyceae). This paper refers mainly to the green algae and diatoms. Green algae include about 8000 species, covering both marine and freshwater environments and contain complex long-chain sugars (polysaccharides) in their cell walls. These carbohydrate cell walls account for a large proportion of the carbon contained in these organisms, though many species contain quite high levels of various lipids (*circa* 20%, see Table 1) and for some species under certain situations this has been quoted as up to 80% oil by wet weight. Diatoms are a group including approximately 100,000 organisms many of which are marine and dominate the marine phytoplankton. They have silicate cell walls and have been of considerable interest in the area because they can accumulate very high levels of lipid. Diatoms, like many other organisms, use the triacylglycerol lipid molecules (TAGs) as energy storage molecules that can be easily transesterified to biodiesel, but a large percentage of the lipids contained in diatoms are phospholipids which are structurally dissimilar to TAGs and do not convert well to biodiesel using traditional trans-esterification procedures. Diatoms also produce a significant amount of a storage carbohydrate called chrysolaminarin, a β-(1-3)-linked glucan molecule. Coccolithophores, that have calcareous external plates called coccoliths, also include some single-celled flagellated algae and are also important in natural oceanic carbon capture.

Lipid, fats and oils and the term 'natural oil' distinguishes those oils produced by biochemical processes from petroleum oils being produced through fossilisation.

Box 2–Favourable characteristics of algae (Benemann, 1997).

It is easy to provide optimal nutrient levels. This is due to the well-mixed aqueous environment as compared to soil and the simple nutrients required.

Related to this is that they can be easily moved around and handled. One can simply pump microalgae where handling of terrestrial crops is more difficult. The aqueous environment does have disadvantages if dewatering is part of the process.

Absence of non-photosynthetic supporting structures (roots, stems, fruit). As most of the microalgae under consideration are single-celled organisms that are self-contained, all are productive and no other tissues have to be supported. Related to this the cells do not have to spend energy moving storage molecules like starch around between tissues.

Continuous production avoids establishment periods of conventional plants. Although some microalgae undergo sexual cycles under some conditions, most often they are grown vegetatively where simple cell fission occurs. This means that under optimal conditions biomass can continuously increase from very low levels without the lag seen with many terrestrial crops.

Ability to adjust harvest rates to keep culture densities at optimal levels at all times. Especially with the continuous culture systems, such as raceway ponds and bioreactors, harvesting efforts can be controlled to match productivity.

Microalgal research and development is inherently faster than terrestrial species. Related to their high cell division rate, research is often simpler and can be performed several orders of magnitude faster with microalgae than with terrestrial crop species. Furthermore, there is substantial evidence that results of small-scale, cost-effective, experiments can be effectively translated to the very large scale that would be required for carbon dioxide capture and/or biofuel production (Sheehan et al., 1998).

Microalgal research and development can spin-off from wastewater treatment and aquaculture efforts. Much can be learned and leveraged from the work done in these other fields.

one would utilise the CO_2 emitted from fossil-fuelled power stations or other industrial sources of CO_2 such as cement processing (Benemann, 1993 1997; Hughes and Benemann, 1997; Vunjak-Novakovic et al., 2005; Greque de Morais and Costa, 2007; Ratledge and Cohen, 2008). There are comparatively few, but valuable, studies in the literature exploring algal capture of either simulated or actual flue gas CO_2 and these are discussed below.

A number of features of algae make them attractive when compared to terrestrial feedstock crops (Box 2). Although their growth requirements are simple (nutrients, a nitrogen and phosphate source, trace metals, water, CO_2 and sunlight) and are similar to terrestrial plants, they use these resources very efficiently (Briggs, 2004) and therefore have high productivity with comparatively low water use (Brown and Zeiler, 1993; Chelf et al., 1994). Fig. 1 is a simple demonstration of how biomass generation is dramatically stimulated by the presence of additional low levels of CO_2. The algae absorb the extra CO_2 present, capturing it as biomass through increased growth. In relation to their potential for capture of CO_2 from fossil power plants microalgae offer additional benefits in that direct CO_2 capture processes are preferable to indirect ones. As microalgae grow in aqueous environments, directly passing flue gases through this medium is a very efficient way of capturing the CO_2 in those streams (Benemann, 1997). The application of CO_2 directly to terrestrial crops via enclosures is likely to be prohibitively expensive though indirect stimulation of land species by flue gases is an alternative approach, which may be cost-effective despite being very much less direct and less efficient. For these reasons many believe that microalgae are the only economic route to biodiesel (Chisti, 2007; Schenk et al., 2008), though there is robust discussion about it (Ratledge and Cohen, 2008).

Currently New Zealand uses about 3.4 billion litres of petrol and about 2.9 billion litres of diesel per year. The Royal Society of New Zealand Energy Panel's report makes a case for bioethanol deployment in New Zealand to replace petrol by 2020 (Watson et al., 2006). This is based on the fact that the technologies for large-scale conversion of lignocellulosic biomass to bioethanol

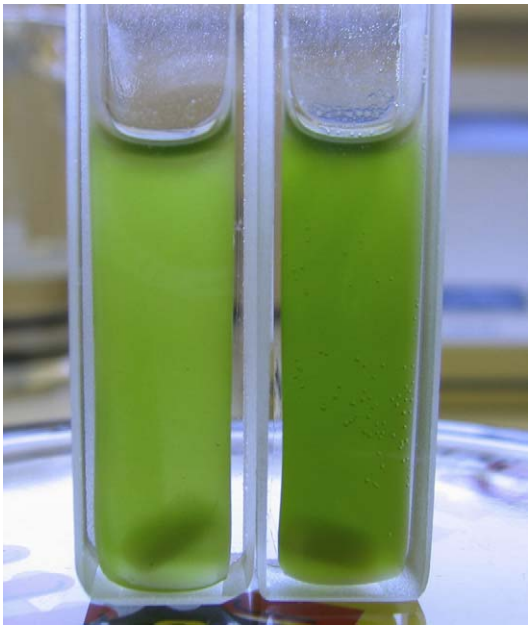

Fig. 1. A simple demonstration of carbon capture as algal biomass. The freshwater *Chlamydomonas reinhardtii* microalgal culture was grown with and without additional CO_2. Cultures were started with identical cell numbers and were not perceptibly green at the beginning of the experiment. The left hand culture was bubbled with air (containing ~0.038% CO_2), the right hand culture was bubbled at the same flow rate with air containing additional 1% CO_2. At 36 h the rapid growth in both cultures is apparent but this growth was greatly stimulated in the presence of the additional CO_2. The difference in biomass was confirmed by measurements of chlorophyll *a* (Chl *a*) showing that twice the biomass is present in the CO_2 culture at 31 μg/ml Chl *a* versus 16 μg/ml Chl *a*.

have matured enough and pilot and pre-commercial scale demonstration projections support their feasibility for rapid uptake. Presently, we do not have the resources to produce enough biodiesel to meet our needs from existing feedstock (mainly tallow at around 200 million litres per year). Microalgal oil could meet this need. The New Zealand Energy Strategy (MED, 2007) calls for a 50% reduction of transport carbon emissions *per capita* by 2040. This is an aggressive target and represents forward thinking. Present New Zealand legislation however states that by 2012 only 3.4% of the energy content of transport fuels must be through biofuels with a slow ramp up from a mandatory ~0.53% from the middle of 2008. This reflects the difficulty of achieving such goals with the present portfolio of biofuel feedstock available in New Zealand (dairy whey to ethanol of approximately 20 million litres per year and tallow to biodiesel (Watson et al., 2006)). It is likely that the 2050 goal will only be met by embracing a variety of emerging technologies. Capturing CO_2 from industrial processes, including power generation, using algae and utilising the biomass generated for our transport needs is one way to help achieve this goal and reduce our overall carbon emissions. Furthermore, worldwide the aviation industry is a significant contributor of global CO_2 emissions (~2% of total global emissions). Because of this there are many efforts, including projects in New Zealand, focused on the production of fuels suitable for transport and aviation purposes from algal biomass.

2.1. Capture of fossil-fuel-driven power plant flue gases

The primary emission in flue gases is CO_2 at between 3% and 15% concentration depending on fuel source and design of the plant; coal-fired plants generally having higher CO_2 emissions. This is an important nutrient for any photosynthetic organism and when delivered to algal cultures in a variety of different ways most studies indicate that CO_2 addition to algal cultures stimulates growth (as demonstrated in Fig. 1). This is true for macroalgae also (Isreal et al., 2005). Other constituents of the flue gases however have to be considered, especially oxides of nitrogen (NOx) and sulphur (SOx) and also metals present at much lower levels, nickel (Ni), vanadium (V) and mercury (Hg), again depending on the fuel used (Table 1). The body of evidence from studies using either gas mixtures that simulate flue emissions (Yanagi et al., 1995; Zeiler et al., 1995; Huack et al., 1996) or actual flue gases (Negoro et al., 1993; Matsumoto et al., 1995, 1997; Maeda et al., 1995; Vunjak-Novakovic et al., 2005) suggest that NOx levels present in flues gases pose no problem for algal growth. The studies also show that the main impact is SOx where the pH of the culture, through the formation of sulphurous acids, can become a problem for some species if the flue gas concentration of SOx is above ~400 ppm (Matsumoto et al., 1997). These studies include a variety of algal species, power plant and fuel-types including coal, some scrubbed for SOx (many of the 'dirtier' plant emissions are already scrubbed using existing but expensive (both in terms of capex and opex) technology to below this level for other reasons such as acid rain prevention), heavy fuel oil, or natural gas and use different methods of application (pulsed or continuous). These data are consistent over different scales and growth systems, from enclosed research and pilot-scale through to large-scale open raceways and some of the studies extend for long periods.

Table 1
Selective emission comparison for combined cycle gas turbine (CCGT) and coal-fired power plants.

Parameter	CCGT (t/GWh)	Coal (t/GWh)
CO_2 emission rate	370	912–1280
SO_2 emmision rate	0.2	0.69–53.94
NOx[a] emission rate	0.4	1.1–4.9
Mercury		0.005–0.064

Data drawn from The US Environmental Protection Agency AP42 Emission Factors Center (http://www.epa.gov/ttn/chief/ap42/index.html) and from NZ resource consent documents. Emissions from coal will vary widely depending on the source of the coal. Contact Energy's Otahuhu B Natural Gas CCGT is a 380 MW power station. It is the largest of its kind in New Zealand and one of the most efficient in the world. It produces around 3150 t of CO_2 per day at about 3% concentration. The exit velocity of the emission stream is less than 20.0 m/s at a temperature of less than 356 K.

[a] Nitrogen oxides.

Nickel and V above 1.0 and 0.1 ppm, respectively, levels that are higher than most flue emissions, decrease algal productivity (Matsumoto et al., 1997) but there is no data to suggest that Hg has any detrimental effects on algal growth and in fact it has been demonstrated that some algae may bio-convert Hg between forms representing a possible route to toxic remediation (Kelly et al., 2007). Bioaccumulation of metals may be important if high-value nutritional oils were the goal (not the focus of this paper), especially from coal-fired plants. Emissions are cleaner from natural gas-fired plants and cleaner still in combined-cycle gas turbines (CCGT; Table 1).

3. Microalgal growth and productivity

A wealth of information is contained in the closeout report of the United States of Department of Energy, Aquatic Species Programme (ASP) (Sheehan et al., 1998). This summarises US$25.05 million of work done by the US National Renewable Energy Laboratory (NREL) over a 20 year period until 1996, mostly on algal growth in open ponds. In contrast the Japanese RITE program from the around the same period concerned highly

engineered PBRs (Murakami and Ikenouchi, 1997). Some designs included solar concentrators and fibre optic delivery of light to different parts of the photobioreactor and showed very high productivity. It is difficult to directly compare figures of productivity for the bioreactors used in these studies with the ponds, as usually productivity per unit area is given for ponds where it is given as productivity per unit volume for enclosed bioreactors. The most useful way to express productivities for comparison between different production methods would be in biomass per unit light energy used or falling over a particular area (Bosma et al., 2007). Few authors do this however and often do not include enough information in their papers to derive this from presented data. The potential of enclosed bioreactors can be demonstrated in that many incorporating artificial lighting show huge productivity. The highest reported is 9.2 g L^{-1} d^{-1} dry weight biomass for a culture of the marine green algae *Chlorococum littorale* at 20 g L^{-1} density for a flat-plate bioreactor with very high intensity artificial lighting (Hu et al., 1998). Remarkably, this reactor could achieve algal culture densities of 84 g L^{-1} and although sunlight energy per unit area would be limiting for *de novo* fuel production, such a system may be useful for CO_2 capture and production of higher value products and in addition higher culture densities mean less energy consumed for downstream dewatering depending on the process the biomass is to be used for (Section 6.2). For enclosed bioreactors utilising sunlight, productivity per unit area is also useful. The commercial bioreactor supplier AlgaeLink claim year round productivity of several different species of algae in the order of 365 t ha^{-1} yr^{-1} for one of their systems. Greenfuel Technologies Corporation, based in Massachusetts USA, who have several large-scale pilot plants operating and focus on CO_2 capture from industrial emitters, demonstrate dry weight productivities between 250 and 292 t ha^{-1} yr^{-1} in their sunlight-powered algal bioreactors. The ASP programme closeout report states open ponds, by the end of the research in 1996, were able to achieve a peak performance of 'almost' 300 t ha^{-1} yr^{-1} dry weight biomass production, whereas at the beginning of the programme they were producing around 50 t ha^{-1} yr^{-1} biomass (Sheehan et al., 1998). In a recent report describing algal biomass for potential production in New Zealand, Heubeck and Craggs say high rate algal pond (HRP, see below) production with CO_2 stimulation is between 40 and 75 t ha^{-1} yr^{-1}(Heubeck and Craggs, 2007).

Large-scale open ponds previously investigated had lower productivity than required for economic deployment, probably due to low night temperatures in the areas where these open ponds were tested. In New Zealand we have a relatively benign climate compared to the central US desert regions. The coupling of waste heat from power plants and other industrial sources might also help to overcome this problem. There are current discussions of the economics of biodiesel production in the recent review by Chisti (Chisti, 2007) who suggests about 1.5–3 times higher productivity is required "the desired levels of cost reduction are substantial, but attainable" (Chisti, 2007).

A widely stated claim is that microalgae are capable of producing 30 times the amount of oil per unit area of land, compared to terrestrial oilseed crops (Sheehan et al., 1998). The actual global oilseed crop (sum of soy, rapeseed, sunflower and palm) oil production in 2007/2008 was 0.592 t ha^{-1} for that year (Yu, 2008). If one assumes an oil concentration in algae of ~42% (for the green algae *Chlorella sp.*, microalgal oil content values are given in Table 2 and there is a table with values of similar magnitude present in Chisti (Chica et al., 2005)), the 365 t ha^{-1} yr^{-1} productivity in the AlgaeLink bioreactors equates to 153.3 t ha^{-1} yr^{-1} oil produced, which is about 259 times better productivity than the actual terrestrial oilseed crops. For open ponds, such as the peak performance of the ASP ponds at ~300 t ha^{-1} yr^{-1} dry weight producing an algae that is say 20–30% oil, production is 100–150 times greater and for continuous HRPs, again assuming 20–30% oil content for the species that dominate them, 17–25 times. On balance therefore the 30 times more productive claim is well justified with regard to oil production. Productivity over terrestrial crops is likely to be higher still for total biomass as opposed to oil, so for fuels not dependent on lipid extraction it is clear that algal production has a huge advantage concerning land use. High algal productivity is in part due to their inherent biochemistry and photosynthetic efficiency.

Table 2
Microalgal lipid content % dry weight (Becker, 1994; Moheimani and Borowitzka, 2005).

Species	Lipid % dry weight
Botyococcus braunii	80
Chlorella protothecoides	57.9
Nannochloris sp.	30–50
Pleurochrysis carterae	30–50
Chlorella pyrenoidosa	46.7
Scenedesmus dimorphus	16–40
Prymnesium parvum	22–38
Dunaliella tertiolecta	35.6
Hormidium Sp.	38
Chlorella vulgaris	14–22
Tetraselmis sueica	20
Euglena gracilis	14–20
Scenedesmus obliquus	12–14

3.1. Microalgae and macroalgae photosynthetic efficiencies versus terrestrial plants

For the purposes of this paper photosynthetic efficiency can be simply defined as the percentage of incident radiation that is converted into biomass. As already mentioned the first step of light capture by photosynthetic LHC in the antennae of plants is extremely high at around 95% (Engel et al., 2007). Later steps are less economical though still in the order of 50%, and actual conversion of solar energy to biomass is in the order of 1–8% (Kruse et al., 2005). This is supported through measurements of the total solar energy conversion efficiency of the cyanobacteria *Spirulina* in open ponds at about 3%, reaching peak at around 4% (Benemann and San Pietro, 2000). It is important to note that for most terrestrial crop species the solar conversion efficiency is in the order of 1%. The ASP closeout report also makes the statement, in the context of oil prices being ~US$13 per barrel at the time, that for algal biodiesel generation to be economic 18% photosynthetic efficiency would have to be reached using microalgae containing 60% lipid (Hill et al., 1984; Sheehan et al., 1998). Photosynthetic efficiencies of 20% have been achieved in open pond or raceway cultures of *Chlorella sp.* (Tamiya, 1957), *Phaeodactylum tricornutum* (Acien Fernandez et al., 1998) and *Tetraselmis suecica* (Laws et al., 1986), but whether this is possible for high lipid species with lower productivity content is uncertain. However the entire economic argument strengthens as oil prices rise.

That algae absorb light very efficiently to then only waste it at later steps is puzzling, but can be explained by competition for resources at the individual cell level. This has led to efforts, which seem counterintuitive at first, to reduce the size of LHCs. The idea is to allow an algal cell to absorb the light it needs but allow the remainder of the light to penetrate further into the culture to support biomass production in its neighbours. This was actually recognised long ago by Vanevaar Bush (Bush, 1953), and in the last few years has been pursued with vigour using modern molecular

genetic methods especially with the aim of producing hydrogen (Polle et al., 2002). Another benefit is that this approach would also limit photo-oxidative damage to the photosynthetic reaction centre. Where direct modification is not possible, such as in countries with low tolerance for genetically modified organisms (GMOs), screening for natural mutants may allow a pathway forward if LHC mutants were necessary for overall economic success. Other reviews summarise the kinds of genetic improvement using recombinant molecular techniques that could help, such as modifying metabolic processes to increase biomass and lipid productivity or partitioning (Chisti, 2007; Huntley and Redalje, 2007; Sheehan et al., 1998).

3.2. Algal metabolism and the biochemistry of lipid production

Microalgae are eukaryotic cells Technically this means they contain a nucleus and other membrane-bound organelles making them structurally more complex than bacteria. This also means they use sophisticated control mechanisms and post-translational biosynthetic processes, yet despite this they have the flexible metabolic repertoire of bacterial microorganisms. This flexibility affords a greater choice and speed of response in metabolic approaches to different situations. An example is that many microalgal cells possess the enzyme pyruvate formate lyase, which is widespread in bacteria, but seldom found in eukaryotes (Hemschemeier and Happe, 2005). This enzyme is key in bacterial fermentation pathways and its presence in microalgae allows fermentative behaviour when oxygen is low (this is important in the metabolism of hydrogen production by microalgae). There is evidence that metabolic pathways involving the enzyme glucose-6-phosphatase (G6P) and ATP trans-esterification behave differently in algal cells partly contributing to their greater productivity over terrestrial species (Woodward et al., 1996, 2000). Another possible contributing factor to algal efficiency is the way they actually fix carbon. There are three main carbon fixation mechanisms employed by plants; the C3, C4 and crassulacean acid metabolism (CAM, which is normally only used in drought-tolerant plants like cacti). CAM and C4 fixation are considered more sophisticated pathways than the simpler and more ancient C3 carbon fixation machinery that most plants use. Both CAM and C4 pathways overcome the tendency of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo, the first enzyme in the Calvin cycle) to waste energy by using oxygen to break down carbon compounds to CO_2. C4 plants do this by separating RuBisCo from atmospheric oxygen, often by fixing carbon in different cell types to cells that contain the rest of the Calvin cycle enzymes. Algae, because of their ancient origin and single-celled nature, have always been thought to rely on C3 carbon fixation, but there is evidence in marine diatoms that the C4 pathway is functional and important (Reinfelder et al., 2000, 2004).

Over-production of the diatom enzyme acetyl CoA carboxylase (ACCase), which catalyses a key metabolic step in the biosynthesis of lipids, did not lead to increased oil production (Sheehan et al., 1998). More recently the squalene synthase gene (BSS) was cloned from the green algae *Botryococcus braunii* and over-expressed in the bacterial cell *Escherichia coli*, a common laboratory organism that is used widely for molecular biology over-expression work. The gene was expressed but failed to have activity in the foreign environment of the bacterial cell (Banerjee et al., 2002). These results are not too surprising as enzymes seldom work in isolation. It is more likely that whole pathways would have to be upregulated and metabolic decision points identified and manipulated.

Observations of intracellular oil droplet-formation under stress and especially nutrient deprivation, lead to much effort trying to find a 'lipid switch' or 'lipid trigger' where a simple manipulation might be able to greatly increase oil biosynthesis. Nutrient stress, especially nitrogen and also silicate for diatoms, was shown to greatly increase oil production in microalgae but at the expense of total biomass production. This can be explained in that the stresses lead to decreased cell division, nitrogen being important for protein biosynthesis and silicate involved in the cell wall structures of diatoms, and in the absence of increasing numbers of cells the microalgae stored up extra lipid reserves. So, as always, one must balance biomass production with high oil yield.

Oil production in higher plants is quite well understood and it has been assumed that the pathways are similar in microalgae though they are less well studied. However, as discussed, nutrient deprivation produces major effects on the quantity and quality of lipids in microalgae where it does not seem to in terrestrial plants and so there are likely to be significant differences in the biochemical pathways. This can be explained to some degree by the single-celled nature of the microalgae, versus terrestrial plants, and how they are able to cope with such stresses as a result. There is though a reasonable understanding on biosynthesis of the unsaturated hydrocarbons in some algal species such as *Botryococcus sp*. This species has received a great deal of attention due to its extremely high levels of oil production, quoted in the literature as being up to 80% of biomass wet weight. This research is reviewed well for the nonisoprenenoid alkadienes and trienes, lycopadienes and unusual isoprenoids (botryococcenes) found in different strains of *Botryococcus braunii* in Banerjee et al. (2002).

Regarding the qualitative differences of microalgal oil versus terrestrial plants, many algae contain high proportions of long chain fatty acids (i.e., C-20, C-22) with a high degree of unsaturation (20:5). These very long chain-polyunsaturated fatty acids (VLC-PUFAs) are important in aquaculture applications as they improve the nutritional quality of feed for fish and shellfish, and have nutritional and nutraceutical applications for humans.

4. Different cultivation methods: ponds and bioreactors

Enclosed bioreactors and open pond are the two predominant methods for growing algae. Each has advantages and disadvantages, but the major difference between the two is the trade-off of cost versus control. Bioreactor systems, although achieving much higher biomass production and having a smaller footprint, are an order of magnitude more expensive than ponds. It has been estimated that even adding simple plastic sheeting to cover over ponds would much more than double total systems capital and operating costs (Benemann, 1996). For bioreactors when comparing batch or continuous systems, only continuous systems are realistically feasible for microalgal biomass (Banerjee et al., 2002). The different types and economics associated with each are reviewed well (Chisti, 2007; Weissman and Goebel, 1987). Bioreactor designs vary widely, but there is room for improvement to make systems simple and cheap enough to be scaled while maintaining the higher productivity and control over the culture than ponds allow. Clearly, an integrative approach where all nutrients are recycled and co-products are generated would be necessary for either ponds or bioreactors to be economic (Chisti, 2008), though this would be easier to achieve for bioreactors because of the greater control over them.

There are many different pond designs; the best for developing algal biomass are shallow raceway ponds that allow efficient light exposure to the population of algal cells, and good mixing. These HRPs are continuously drawn from and have a retention time in the order of a few days to a week. Impressively the "Outdoor Test Facility (OTF)", an unlined raceway pond described in the ASP, was designed in such a way to have a total power consumption of

0.04 W m^{-2} (only 40 W for the entire 1000 m^2 pond) employing a counter-current injection system that resulted in 100% efficient CO_2 transfer to the water with utilisation of over 90% of CO_2 (Weissman and Goebel, 1987).

There are several huge ponds (in the order of 50 ha total area) in Australia for mass culture of the cyanobacterium *Spirulina (Arthospira) platensis*. These are essentially artificial lagoons that are very shallow (~20 cm deep) and constructed either on the bed of a hypersaline coastal lagoon, or formed by artificially expanding a lagoon (Belay, 1997). While these types of unmixed ponds have no energy input, being essentially hands off, they are not suitable for CO_2 stimulation of growth and have very low levels of overall productivity compared with raceway designs. They also suffer from evaporation problems and are therefore mostly suitable for hypersaline tolerant species.

Open mass culture ponds cannot be sterilised or kept under axenic conditions and therefore if specific species of microalgae are required (such as those with high productivity or high lipid content) then large inoculums, or starter cultures, are needed to get the required cells to levels high enough to dominate. Literature data supports the approach of continuously generating high levels of inoculating cultures for open ponds using smaller bioreactors, comparing the economics of the bioreactors coupled to ponds versus larger enclosed bioreactors on their own (Huntley and Redalje, 2007). An alternative approach is the choice of algal species that have selective and demanding environments such as *Dunaliella sp*. which requires high salt conditions and out-competes others in this environment. Often native or local species of microalgae dominated.

Enclosed reactors do offer the advantage of having easier control of the type of algae growing, particularly important to reach economic productivities, depending on desired products. One other feature that could be applied to enclosed bioreactor designs the possible advantage of immobilised algal cells over suspension. The benefits of having microbial cells fixed to the surface are well known especially in the fermentation industry. These include protection against shear forces, encouraging production and possibly less energetic dewatering of biomass. However this technology might be best for extra-cellular products like ethanol or butanol and has not been explored extensively for oil production, which is produced intra-cellularly. The approach might be applied for products that are continuously extracted from the culture, an area that is relatively new but gaining a great deal of interest.

5. Oceanic algal carbon sequestration

There has been a great deal of analysis done on the amount of land needed for production of microalgae for biofuels production in the United States (Briggs, 2004; Chisti, 2007; Huntley and Redalje, 2007). Although most of these studies are in the context of using North American saline aquifers, it is sufficient to say that these analyses suggest that there is certainly more than enough non-arable land suitable for mass algal cultivation for biofuel production to meet the needs of that country. Drawing from these studies it is also probable that in New Zealand there is enough land that does not compete with food production that is also close to industrial CO_2 sources to meet our liquid fuel requirements, but the actual analysis for different algal crops to specific biofuels has not yet been completed. A major difference in the New Zealand situation is that we do not have the extremely large saline aquifers that are present in the United States and Australia. New Zealand does however have an approximately 4 million square kilometer oceanic exclusive economic zone (EEZ) under its control. This is soon to be extended to ~5.7 million square kilometers, extending to the edge of the continental shelf surrounding the country. Using satellite data estimates for both chlorophyll *a* (Chl *a*) and carbon and integrating these into established models (Behrenfeld et al., 2005) Cawthron biophysical oceanographer Ben Knight has calculated the productivity of New Zealand waters temporally and spatially (Knight and Jiang, 2008). Using the mean of two different vertically generalized production model (VGPM) estimates, this work shows that approximately 650 teragrams of carbon per year is fixed by microalgae in our present EEZ. This equates to ~105 times New Zealand's carbon consumption as liquid transport fuels.

At a physical level the ocean has already absorbed nearly half of the anthropogenic CO_2 generated since the industrial revolution and this has caused a measurable effect on the acidity of the water, which is negatively affecting marine life including corals and carbonate-containing microalgae (Riebesell et al., 2007). At a biophysical level however, it has been suggested that increased levels of CO_2 on the atmosphere might actually stimulate a 'biological pump' involving growth of some species of algae for the transport of carbon to long-term deep ocean storage (Arrigo, 2007; Riebesell et al., 2007). The capacity of this process however is unknown at this time.

In the natural carbon cycle some oceanic species of microalgae such as the diatom *Chaetoceros spp.* and coccolithophores like *Isochrysis spp.* and *Pavola spp.* naturally sink to eventually become fossilised. Dead microalgae coalesce to form semi-solid structures called transparent exopolymer particles (TEPs) which are sticky and facilitate the aggregation and increased sinking of other organic particles transporting carbon to deep water (Arrigo, 2007). These observations have led to the possibility that these natural cycles might be enhanced. There are two mechanisms proposed. One is the large-scale use of oceanic pumps to cause upwelling of nutrient-rich water to surface waters for stimulating microalgal production (Lovelock and Rapley, 2007). This effect has been demonstrated on a small scale (Aure et al., 2007), but the environmental impact of the truly huge structures required seems questionable. The second approach stems from the understanding that natural algal growth is maximal where terrestrial sources bring nutrients to the sea. Of note were the pioneering experiments in the Southern Ocean stimulating microalgal growth with iron fertilization (Boyd et al., 2000). To date there have been at least eleven mesoscale experiments of this nature and several private ventures are now seeking gain from selling carbon credits using these techniques (Kintisch, 2007). The biggest problem facing for carbon sequestration scheme is that obviously the time scale does not allow for fossilisation and there is not enough data about what the accelerated carbon transport is doing to the less than well-understood deep-sea ecosystem and how long it remains in the deep sea. Anaerobic dead zones, methane production and associated marine life die-off possibilities. It is clear that further experimentation is needed with refined measurements to understand the process and this should be done before it is used as a global tool for addressing climate change.

6. Utilising or storing the biomass: algal harvesting

An alternative to stimulating natural biogeochemical cycles is to produce carbon-containing products from the biomass generated (whether this be oceanic or in dedicated production facilities directly coupled to sources of anthropogenic CO_2 such as industrial emissions), that can be more easily and safely stored in geological reservoirs. True sequestration requires capture into materials that will not release the carbon dioxide back into the atmosphere. It is ironic that oil products, 'biocrude', might be the most suitable for this and it is technically feasible to use biological

and chemical methods for producing it. It is extremely unlikely however, in the present global economic market that this would happen. Other examples include conversion into biochar, a charcoal produced from biomass in various ways, that can be used as a soil conditioner, as practiced by pre-Columbian Amazonian Indians, but is also suitable for deep-buried storage of carbon. Incorporation into building materials is another route; once the materials have reached the end of their useful life they can either be recycled or buried. This can be by production of carbon-based polymers that can be extracted from the algae and used to make the materials or even simply incorporating algal biomass into building material such as plastics or concrete has been explored (Usui and Masahiro, 1997). Another scenario is the carbon is recycled as fuels where the biomass is processed into biofuels that lessen the demand for new fossil release from geological reservoirs, and for this to be made economic via co-products ranging from nutritional oils to industrial chemicals (Dodds and Gross, 2007). The simplest way to recycle carbon from biomass is to through co-firing of that biomass with other fossil fuels (Hughes and Benemann, 1997). However, the aquatic nature and the required energy input to dewater algal biomass to achieve this means that it is not as suitable for this purpose as other biomass sources such as wood waste.

6.1. Harvesting methods

There are many methods available for harvesting algae. Cells are more dilute in pond cultures versus bioreactor cultures and natural oceanic microalgal levels are one to two orders of magnitude lower still. The method chosen depends greatly on the final product and the processes subsequently used: some processes require the algae to be completely dewatered, whilst others may require moderate dewatering. The presence of other chemicals such as flocculants must also be taken into consideration. Filtration is most commonly used. Micro-strainers, rotating screen filters with a backwash, are widely used for collecting algae such as *Spirulina* but it is unlikely that this would be economic for collecting algae for non-food products.

Flocculation, where multivalent cations are added to overcome the overall negative charge carried on the surface of most microalgae that normally prevents them sticking together in suspension, is a relatively low-cost method (Eisenberg et al., 1981). It is often combined with dissolved air floatation (DAF). Both are mature technologies used in sewage ponds and wastewater treatment. DAF uses tiny bubbles that are injected under high pressure into the water column and as they rise to the surface they drag organic molecules and cells with them. Efficiency is increased using flocculants but their addition can cause problems, depending on the how the biomass is to be processed downstream.

Collection of algae by centrifugation is only really feasible for relatively high value products (Molina Grima et al., 2003), though continuous centrifugation has been explored which might be more economic if systems are built on a large scale (Briggs, 2004). A variation of centrifugation that has not been explored a great deal for use with algal biomass collection, but is widespread in the petroleum and mining industries, is the use of hydrocyclones. In these devices water containing the particles, in this case algal cells, is channelled in a spiral fashion creating centripetal forces causing the denser particles to be spun out of the traversing liquid. Although the technique works for removing dense particles from liquid streams and for separating oil from water, their application to soft algal cells is experimental. However, their simplicity and fewer moving parts avail their potential for large-scale economic application.

6.1.1. Extracting lipid from algal biomass

There are several approaches to extracting lipid from harvested algal biomass, including solvent extraction, osmotic shock, ultrasonic extraction and critical point CO_2 extraction.

Hexane is the solvent typically used, either alone or in combination with an oil expeller or press. After the oil has been extracted using an expeller, the leftover pulp can be mixed with cyclohexane to extract the remaining oil. The oil dissolves in the cyclohexane, and the pulp is filtered out from the solution. The oil and cyclohexane are separated by means of distillation. These two stages (cold press and hexane solvent) together are able to derive more than 95% of the total oil present in the algae.

Osmotic shock is a sudden reduction in osmotic pressure causing cells to rupture and release cellular components including oil. Some marine species of algae such as *Dunaliella sp*. that lack a cell wall are particularly suitable for this. They optimally grow in hypersaline conditions and then can be easily ruptured by diluting in non-salty water (Williams et al., 1978). Ultrasonic extraction can greatly accelerate extraction processes. Ultrasonic waves create cavitation bubbles in a solvent and when these bubbles collapse near the cell walls the shock waves developed cause cell walls to break and release the oil into the solvent.

Critical point gas/fluid extraction is probably the most efficient method for complete extraction of the oils. The use of CO_2 for this is the most mature technology and the CO_2 is recycled during the process. This is a very energy-intensive process however and it is unlikely to be economic for the extraction of commodity oils for biofuels.

Relatively low-grade heat (such as waste heat) could be employed to separate solvents from oil in some circumstances, greatly increasing the overall economics. Osmotic shock, though requiring low-energy input, is probably the method with the lowest efficiency and creates a further issue for some downstream processes, as water content can be a problem requiring energy input to overcome. In one project under the ASP, solvent extraction costs were three times higher for algal oil than for soybean oil due to high moisture content of the paste in the experiment (Sheehan et al., 1998). Mechanical dewatering (pressing and filtration) can be cheaper than heating (Molina Grima et al., 2003), but the real key is to have as few steps as possible and simple scalable extraction.

6.2. Processing biomass to biofuels

Whether harvesting of microalgal biomass from the ocean (which would require breakthrough technology to make it economically viable and sustainable), or from bioreactors and ponds there are several approaches for processing it; chemical, biological, thermolytic and non-lethal extraction or ‘milking’. Until recently most interest has been in lipid-producing algae, where the lipids can be extracted and chemically processed to biodiesel. The most straightforward chemical conversion involves the transesterification of triacylglycerides (TAGs) to biodiesel. As discussed earlier some microalgae produce high levels of TAGs but these are often varieties that grow slowly. Furthermore, many marine species produce higher levels of phospholipids than TAGs, which do not behave optimally during transesterification and there is increased interest in these marine species for their ability to grow in the saline aquifers as fresh water becomes increasingly precious. There is also the issue of what to do with the remaining biomass that would be generated on a large scale. Already there is a glut on the world market for glycerol, a by-product of transesterification of TAGs, because of the increased biodiesel production from terrestrial crop oils. This has been used in the soap industry in the past but the increased amounts being

presently produced are not being absorbed in this way, representing the difficulty of economics of co-products matching the scale of commodities like transport fuels. New ways of using this by-product, such as for plastic production are becoming economic. Proteinaceous and polysaccharide (long chain complex sugars) remnants can be used in a variety of ways, the integrative approach of consuming this in anaerobic digesters is one pathway (Chisti, 2008) and protein-rich residues can be used as fertilizers.

Two approaches avoid some of these difficulties by turning more than just the lipid biomass into biofuels and therefore might be more suitable for more productive species of algae. (i) Biological conversion includes fermentation, yielding products such as ethanol and butanol. Yeast fermentation of simple sugars to ethanol is limited by having to produce simple sugars from the algal biomass, a similar issue to those seeking to produce ethanol from woody biomass. However, other organisms might be more suitable. Many clostridial species are much more flexible, being able to convert a wide range of substrates to useful biofuels such as butanol (Biebl, 2001; Nakas et al., 1983). A promising approach to decrease processing steps would be to use high-productivity marine algal species like *Dunaliella*, where osmotic shock with fresh water would liberate all the cellular constituents making them available and at the same time adjust the salt concentration to be suitable for clostridial fermentation. New approaches would be required to continuously remove the butanol from preferably a continuous fermentation, but these have been suggested (Jones and Woods, 1986) and are doable with today's technology. (ii) Thermolytic techniques too, offer much promise for the wholesale conversion of total algal biomass to biofuels. In its pure form thermolysis includes traditional pyrolysis where biomass is converted to biofuel in the absence of oxygen, but this is very energy-intensive and might produce small amounts of potentially toxic by-products. There are several projects worldwide developing less extreme thermolytic processes that combine a chemical transformation with less severe heat that overcome some of these problems (Huber et al., 2005, 2003; Rostrup-Nielsen, 2005).

The last process takes a counter approach from trying to use all the biomass generated by just extracting the molecule of interest. Non-lethal extraction, or 'milking' the algae, for specific molecules has the potential advantage of greater efficiency in conversion to product because only the molecule of interest is being removed from the cell, which can then go on to make more product. Fewer nutrients are needed and production speed is increased as new cells do not need to be generated for the next production cycle. In one article there was a description of hexane being used to extract TAGs from *Botryococcus braunii* and repeated exposures not affecting growth and continued productivity (Baker, 2005). Carotenoids, high value lipids, have also been selectively extracted from the green alga *Chlorella sp.* using decane (Hejazi et al., 2004). If appropriate engineering solutions are developed and even more specific targeting of useful molecules achieved, this approach may improve the environmental impact of using oceanic microalgae.

7. New Zealand policy and regulatory environment

New Zealand's policy environment has been hailed globally in biotechnology circles as "a transparent, enforceable, publicly accessible, and scientifically robust regulatory framework" (Herrera, 2005) allowing clarity of status for biotechnological pursuits. The basis for this is the Resource Management Act (RMA) and a framework for recognising the rights and sensitivities of indigenous peoples. Facing the issues of GMOs, the Hazardous Substances and New Organisms (HSNO) Act was passed in 1996, with a new regulatory agency called the Environmental Risk Management Authority (ERMA) setup to administer it. In 2003 the country amended many regulations to ensure that GMO research was open and performed safely, whilst respecting Māori cultural traditions. The framework installed affects all projects, including non-GMO efforts that might impact on the environment, such as large-scale algal growth or harvesting.

Although the legislative landscape is not perfect, in New Zealand legal action does not paralyse research and development as it does in many countries, where anti-biotech groups can stall projects through the legal system or win injunctions based on claims of possible harm to the environment. Once approval is obtained, usually after extensive public consultation and any concerns being addressed before consent is granted, then the project is able to proceed with surety. Compliance is assured through regular checks and public transparency maintained via updates on the ERMA website. This clarity has attracted biotechnology developers and companies to New Zealand where they see the country's regulatory protocol as extremely robust and politically legitimate. Biosecurity New Zealand (BNZ), a division of the Ministry of Agriculture and Forestry (MAF) enforce the RMA and are responsible for importation of new species.

7.1. Working within the HSNO act

For projects concerning this paper such as large-scale growth of microalgae (considered to be cultures over 10 L) or algal harvesting, different procedures are to be followed depending on whether the organism is considered 'new', at the species level, under section 2A of the HSNO act. This is important for developers seeking to bring microalgal strains with them for projects in New Zealand and for those that are, or are seeking to, produce GMOs in some way, such as LHC mutants. There are several resources available for doing this. ERMA maintains a database that is searchable at http://www.ermanz.govt.nz/no/ and there is also an 'unwanted organisms' list that can be searched at the BNZ website at http://www.biosecurity.govt.nz/commercial-imports/unwanted-organisms-register/search. According to the act an organism is new if it is not listed on a containment approval, it is genetically modified, or it was not present in New Zealand immediately before 29 July 1998. If an organism is not new, the HSNO Act does not regulate it and approvals are not needed to import, release or propagate it, except to genetically modify it, or if it is has some eco-toxic properties or is part of a product being used for those properties. Regardless of whether an organism is new or not, it is also likely that a permit to import it is required to be obtained from MAF for species being brought into the country. Sometimes MAF can impose conditions on the permit on a case-by-case basis.

If evidence suggests the organism is not new, application to ERMA for a 'determination of status' is necessary which can be done through two routes, either non-statutory or statutory. One can take the non-statutory route if the evidence is clear and indisputable, and the organism is not listed on a containment (or conditional release) approval. Evidence of this needs to be provided and there is no fee for this pathway. The result is a letter advising of the status of the organism, which is often also copied to the MAF imports group and is advisory only and may not be recognised in a court of law. If the evidence is not equivocal or if the organism is currently listed on a containment approval and the decision needs to be reversed, then the statutory route needs to be taken. There is an application fee (currently $1125.00NZ) and a submission that is considered by an ERMA committee, after which a decision is made and publicly notified. For this reason, some practitioners prefer the statutory route for legal clarity.

If the organism is new, it is regulated under the HSNO Act, and approval is needed to import, develop, store, or in any other way

use it for the project. Importing algae for use in large-scale bioreactors or ponds normally fits under the definition of a 'development', which can be either indoor or outdoor (both in containment, more information following). Both 'development in containment' and 'import in containment' applications have a similar process where the appropriate form is completed and fee paid (currently $2250NZ) and a decision made by an ERMA committee within 30 days and again made publicly available. Advice is normally sought directly from ERMA about which is most suitable on a case-by-case basis as is the necessity for Māori consultation. One difference between the two applications is that containment approvals can be used by anyone with suitable containment facilities, unless the approval states that it is limited to the applicant. Development approvals on the other hand are solely limited to the applicant. There is a third type of application where one applies to have the organism released (i.e., no containment). The burden of information is much higher for a full release and the fee is currently $33,750.00NZ. It is also likely that Import Health Standards from MAF are required to be met in this situation. To date there have not been any release applications for algae, so this would break new ground.

Containment refers to both the facility and the practices used for handling the organism and these are outlined and approved by MAF under a series of containment standards. Algae are covered under the 'Facilities for Microorganisms and Cell Cultures' standard, available at the BNZ website http://www.biosecurity.govt.nz/border/transitional-facilities/animals/154-03-02.htm.

Regulations on large-scale production of organisms that are not new, are not regulated by ERMA but are still covered by the RMA, normally the local authority such as a regional council or territorial authority, depending on the scale of production. Here a 'resource consent' needs to be obtained as the activities are unlikely to be part of the district or regional plan (if they were included they would be allowed 'as of right'). For the kinds of projects relevant here it is most likely that MAF containment regulations are required to be adhered to. There are several categories that might be relevant including; land use consent, water permit, discharge permit or a coastal permit. The consent application deals with social, cultural and ecological considerations, significance of effects and place of community values. Resource consent may also be granted with a set of conditions that need to be complied with in order to ensure minimal environmental impact.

7.2. Regulations regarding oceanic fertilization

With the increased interest, especially from commercial ventures, in oceanic fertilization schemes, it is unclear who would be responsible. Within the New Zealand EEZ presumably resource consents as described above would have to be gained. For those projects outside these areas, in the open ocean or in other countries, there is no precedent for this kind of intervention. The closest international agreement is the Convention on the Prevention of Marine Pollution by Dumping of Wastes and Other Matter 1972, commonly called the "London Convention", "London Dumping Convention", or "LC '72" (also abbreviated as "Marine Dumping"). This aims to control pollution of the sea by dumping, covering the deliberate disposal at sea of wastes or other matter from vessels, aircraft, and platforms. There are 81 countries that have signed the convention. It is unclear however, whether fertilization could actually be considered dumping. Despite this, representatives from the convention have stated "given the present state of knowledge regarding ocean fertilization, such large-scale operations are currently not justified".

8. Summary

Despite the very real promise in algal technology for carbon recycling and biofuel production there is much basic science to be done to allow its deployment. This includes integration of various steps on the algal value chain for overall economic success, such as coupling the process with nutrients from wastewater and using waste heat, and designing fuel production processes where less dewatering is necessary. Breakthroughs are required in harvesting technologies and in economic scaleable bioreactor design and materials.

The capture of CO_2 from industrial sources like fossil-fuelled power plants represents carbon recycling, not mitigation. This results in more efficient use of these fossil resources and thus goes towards mitigating the problem to some degree. It is interesting to note that over ten times more CO_2 is fixed by plants into biomass, and annually released by decomposers and food chains, than is emitted to the atmosphere due to the burning of fossil fuels (Hughes and Benemann, 1997). It is possible that algal capture of CO_2, might one day also lead to technology that in the right economic climate is able to return carbon to the large carbon sinks, re-linking anthropogenic use to the carbon cycle.

References

Acien Fernandez, F.G., Garcıa Camacho, F., Sanchez Perez, J.A., Fernandez Sevilla, J.M., Molina Grima, E., 1998. Modeling of biomass productivity in tubular photobioreactors for microalgal cultures: effects of dilution rate, tube diameter, and solar irradiance. Biotechnol. Bioeng. 58, 605–616.

Arrigo, K.R., 2007. Carbon cycle: marine manipulations. Nature 450, 491–492.

Aure, J., Strand, O., Rune Erga, S., Strohmeier, T., 2007. Primary production enhancement by artificial upwelling in a western Norwegian fjord. Mar. Ecol. Prog. Ser. 352, 39–52.

Baker, G., 2005. Ironsands to the rescue, DEMM.

Banerjee, A., Sharma, R., Chisti, Y., Banerjee, U.C., 2002. *Botryococcus braunii*: a renewable source of hydrocarbons and other chemicals. Crit. Rev. Biotechnol. 22, 245–279.

Becker, E.W., 1994. Microalgae: biotechnology and microbiology. In: Baddiley, J. (Ed.), Cambridge University Press, Cambridge, New York.

Behrenfeld, M., Boss, E., Siegel, D., Shea, D., 2005. Carbon-based ocean productivity and phytoplankton physiology from space. Global Biogeochemical Cycles 19, 1–14.

Belay, A., 1997. Mass culture of Spirulina outdoors: the Earthrise Farms experience. In: Vonshak, A. (Ed.), *Spirulina platensis* (*Arthrospira*) Physiology, Cell Biology & Biotechnology. Taylor & Francis, London, pp. 131–158.

Benemann, J., 1993. Utilization of carbon dioxide from fossil fuel-burning power plants with biological systems II. Energy Convers. Manage. 34, 999–1004.

Benemann, J., 1996. Hydrogen biotechnology: progress and prospects. Nat. Biotechnol. 14, 1101–1103.

Benemann, J., 1997. CO_2 mitigation with microalgal systems. Energy Convers. Manage. 38, S475–S479.

Benemann, J.R., San Pietro, A.S., 2000. Technical workshop on biological hydrogen production, US Department of Energy Hydrogen R&D Program, Bethesda, MD.

Biebl, H., 2001. Fermentation of glycerol by *Clostridium* pasteurianum–batch and continuous culture studies. J. Ind. Microbiol. Biotechnol. 27, 18–26.

Bosma, R., van Zessen, E., Reith, J.H., Tramper, J., Wijffels, R.H., 2007. Prediction of volumetric productivity of an outdoor photobioreactor. Biotechnol. Bioeng. 97, 1108–1120.

Boyd, P.W., Watson, A.J., Law, C.S., Abraham, E.R., Trull, T., Murdoch, R., Bakker, D.C., Bowie, A.R., Buesseler, K.O., Chang, H., Charette, M., Croot, P., Downing, K., Frew, R., Gall, M., Hadfield, M., Hall, J., Harvey, M., Jameson, G., LaRoche, J., Liddicoat, M., Ling, R., Maldonado, M.T., McKay, R.M., Nodder, S., Pickmere, S., Pridmore, R., Rintoul, S., Safi, K., Sutton, P., Strzepek, R., Tanneberger, K., Turner, S., Waite, A., Zeldis, J., 2000. A mesoscale phytoplankton bloom in the polar Southern ocean stimulated by iron fertilization. Nature 407, 695–702.

Briggs, M., 2004. Widescale Biodiesel Production from Algae. UHN BioDiesel group.

Brown, L.M., Zeiler, K.G., 1993. Aquatic biomass and carbon dioxide trapping. Energy Convers. Manage. 34, 1005–1013.

Bush, V. (Ed.), 1953. Quoted in. Carnegie Insitute, Washington D.C.

Chelf, P., Brown, L.M., Wyman, C.E., 1994. Aquatic biomass resources and carbon dioxide trapping. Biomass Bioenergy 4, 175–183.

Chica, R.A., Doucet, N., Pelletier, J.N., 2005. Semi-rational approaches to engineering enzyme activity: combining the benefits of directed evolution and rational design. Curr. Opin. Biotechnol. 16, 378–384.

Chisti, Y., 2007. Biodiesel from microalgae. Biotechnol. Adv. 25, 294–306.

Chisti, Y., 2008. Biodiesel from microalgae beats bioethanol. Trends Biotechnol. 26, 126–131.

Dodds, D.R., Gross, R.A., 2007. Chemicals from biomass. Science 318, 1250–1251.

Eisenberg, D.M., Koopman, B., Benemann, J., Oswald, W.J., 1981. Algal bioflocculation and energy conservation in microalgal sewage ponds. Biotechnol. Bioeng. Symp., 429–449.
Engel, G.S., Calhoun, T.R., Read, E.L., Ahn, T.K., Mancal, T., Cheng, Y.C., Blankenship, R.E., Fleming, G.R., 2007. Evidence for wavelike energy transfer through quantum coherence in photosynthetic systems. Nature 446, 782–786.
Greque de Morais, M., Costa, J.A., 2007. Biofixation of carbon dioxide by *Spirulina* sp. and *Scenedesmus obliquus* cultivated in a three-stage serial tubular photobioreactor. J. Biotechnol. 129, 439–445.
Hejazi, M.A., Kleinegris, D., Wijffels, R.H., 2004. Mechanism of extraction of beta-carotene from microalga *Dunaliellea salina* in two-phase bioreactors. Biotechnol. Bioeng. 88, 593–600.
Hemschemeier, A., Happe, T., 2005. The exceptional photofermentative hydrogen metabolism of the green alga *Chlamydomonas reinhardtii*. Biochem. Soc. Trans. 33, 39–41.
Herrera, S., 2005. New Zealand: green haven for biotech? Technol. Rev.
Heubeck, S., Craggs, R., 2007. Resource assessment of algae biomass for potential bioenergy production in New Zealand, Bioenergy Options Program.
Hill, A., Feinberg, A., McIntosh, R., Neenan, B., Terry, K., 1984. Fuels from microalgae: technical status, potential, and research issues, Report: Solar Energy Research Institute. Solar Energy Research Institute, Golden, Colorado, pp. SERI/SP-231-2550.
Hu, Q., Kurano, N., Kawachi, M., Iwsaki, I., Miyachi, S., 1998. Ultrahigh-cell-density culture of a marine green alga *Chlorococcum littorale* in a flat-plate bioreactor. Appl. Microb. Biotechnol. 49, 655–662.
Huack, J.T., Olson, G.J., Scierka, S.J., Perry, M.B., Ataai, M.M., 1996. Effects of simulated flue gas on growth of microalgae, 212th ACS National Meeting. American Chemical Society, Orlando, Fl.
Huber, G.W., Chheda, J.N., Barrett, C.J., Dumesic, J.A., 2005. Production of liquid alkanes by aqueous-phase processing of biomass-derived carbohydrates. Science 308, 1446–1450.
Huber, G.W., Shabaker, J.W., Dumesic, J.A., 2003. Raney Ni–Sn catalyst for H2 production from biomass-derived hydrocarbons. Science 300, 2075–2077.
Hughes, E., Benemann, J., 1997. Biological fossil CO_2 mitigation. Energy Convers. Manage. 38, S467–S473.
Huntley, M.E., Redalje, D.G., 2007. CO_2 mitigation and renewable oil from photosynthetic microbes: a new appraisal. Mitigation Adaptation Strategies Global Change 12, 573–608.
Isreal, A., Gavrieli, J., Glazer, A., Friedlander, M., 2005. Utilization of flue gas from a power plant for tank cultivation of the red seaweed *Gracilaria cornea*. Aquaculture 249, 311–316.
Jones, D.T., Woods, D.R., 1986. Acetone–butanol fermentation revisited. Microbiol. Rev. 50, 484–524.
Kelly, D.J., Budd, K., Lefebvre, D.D., 2007. Biotransformation of mercury in pH-stat cultures of eukaryotic freshwater algae. Arch. Microbiol. 187, 45–53.
Kintisch, E., 2007. Carbon sequestration. Should oceanographers pump iron? Science 318, 1368–1370.
Knight, B.R., Jiang, W.M., 2008. Application of Rapid Assessment Technique for Assessing Primary Production Constraints in New Zealands EEZ. Cawthron Report no. 1542.
Kruse, O., Rupprecht, J., Mussgnug, J.H., Dismukes, G.C., Hankamer, B., 2005. Photosynthesis: a blueprint for solar energy capture and biohydrogen production technologies. Photochem. Photobiol. Sci. 4, 957–970.
Laws, E.A., Taguchi, S., Hirata, J., Pang, L., 1986. High algal production rates achieved in a shallow outdoor flume. Biotechnol. Bioeng. 28, 191–197.
Lovelock, J.E., Rapley, C.G., 2007. Ocean pipes could help the Earth to cure itself. Nature 449, 403.
Maeda, K., Owada, M., Kimura, N., Mata, K., Karube, I., 1995. CO_2 fixation from the flue gas on coal-fired thermal power plant by microalgae. Energy Convers. Manage. 36, 717–720.
Matsumoto, H., Hamasaki, A., Sioji, N., Ikuta, Y., 1997. Influence of CO_2, SO_2 and NO in flue gas on microalgae productivity. J. Chem. Eng. Jpn. 30, 620–624.
Matsumoto, H., Shioji, N., Hamasaki, A., Ikuta, Y., Fukuda, Y., Sato, M., Endo, N., Tsukamoto, T., 1995. Carbon dioxide fixation by microalgae photosynthesis using actual flue gas discharged from a boiler. Appl. Biochem. Biotechnol. 51/52, 681–692.
MED, 2007. The New Zealand Energy Strategy to 2050—Powering Our Future. Ministry of Economic Development.
Meier, R.L., 1955. Biological cycles in the transformation of solar energy into useful fuels. In: Daniels, F., Duffie, J.A. (Eds.), Solar Energy Research. Madison University Wisconsin Press, Wisconsin, pp. 179–183.
Moheimani, N., Borowitzka, M., 2005. Culturing Coccolithophorid Algae for Carbon Dioxide Bioremediation, 7th Annual Post-Graduate Symposium. The Royal Society of Western Austrailia, Perth.
Molina Grima, E., Belarbi, E.H., Acien Fernandez, F.G., Robles Medina, A., Chisti, Y., 2003. Recovery of microalgal biomass and metabolites: process options and economics. Biotechnol. Adv. 20, 491–515.
Murakami, M., Ikenouchi, M., 1997. The biological CO_2 fixation and utilization project by RITE (2)—screening and breeding of microalgae with high capability in fixing CO_2. Energy Convers. Manage. 38, S493–S497.
Nakas, J.P., Schaedle, M., Parkinson, C.M., Coonley, C.E., Tanenbaum, S.W., 1983. System development for linked-fermentation production of solvents from algal biomass. Appl. Environ. Microbiol. 46, 1017–1023.
Negoro, M., Hamasaki, A., Ikuta, Y., Makita, T., Hirayama, K., Suzuki, S., 1993. Carbon dioxide fixation by microalgae photosynthesis using actual flue gas discharged from a boiler. Appl. Biochem. Biotechnol. 39/40, 643–653.
Polle, J.E.W., Kanakagiri, S., Jin, E., Masuda, T., Melis, A., 2002. Truncated chlorophyll antenna size of the photosystems—a practical method to improve microalgal productivity and hydrogen production in mass culture. Int. J. Hydrogen Energy 27, 1257–1264.
Rabinowitch, E., 1961. Photochemical utilization of light energy. Proc. Natl. Acad. Sci. USA 47, 1296–1303.
Ramaswamy, V., Boucher, O., Haigh, J., Hauglustaine, D., Haywood, J., Myhre, G., Nakajima, T., Shi, G.Y., Solomon, S. (Eds.), 2001. Radiative Forcing of Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, New York, USA.
Ratledge, C., Cohen, Z., 2008. Microbial and algal oils: do they have a future for biodiesel or as commodity oils? Lipid Technol. 20, 155–160.
Reinfelder, J.R., Kraepiel, A.M., Morel, F.M., 2000. Unicellular C4 photosynthesis in a marine diatom. Nature 407, 996–999.
Reinfelder, J.R., Milligan, A.J., Morel, F.M., 2004. The role of the C4 pathway in carbon accumulation and fixation in a marine diatom. Plant Physiol. 135, 2106–2111.
Riebesell, U., Schulz, K.G., Bellerby, R.G., Botros, M., Fritsche, P., Meyerhofer, M., Neill, C., Nondal, G., Oschlies, A., Wohlers, J., Zollner, E., 2007. Enhanced biological carbon consumption in a high CO_2 ocean. Nature 450, 545–548.
Rostrup-Nielsen, J.R., 2005. Chemistry. Making fuels from biomass. Science 308, 1421–1422.
Schenk, P.M., Thomas-Hall, S., Stephens, E., Marx, U.C., Mussgnug, J.H., Posten, C., Kruse, O., Hankamer, B., 2008. Second generation biofuels: high-efficiency microalgae for biodiesel production. Bioenergy Res. 1, 20–43.
Sension, R.J., 2007. Biophysics: quantum path to photosynthesis. Nature 446, 740–741.
Sheehan, J., Dunahay, T., Benemann, J., Roessler, P., 1998. A Look Back at the US Department of Energy's Aquatic Species Program—Biodiesel from Algae. NREL closeout report.
Tamiya, H., 1957. Mass culture of algae. Ann. Rev. Plant Physiol. 8, 309–333.
Trenberth, K.E., 2001. Stronger evidence of human influences on climate—The 2001 IPCC assessment. Environment 43, 8–19.
Usui, N., Masahiro, M., 1997. The biological CO_2 fixation and utilization project by RITE (1)—highly-effective photobioreactor systems. Energy Convers. Manage. 38, S487–S492.
Vunjak-Novakovic, G., Kim, Y., Wu, X., Berzin, I., Merchuk, J.C., 2005. Air-lift bioreactors for algal growth on flue gas: mathematical modeling and pilot-plant studies. Ind. Eng. Chem. Res. 44, 6154–6163.
Watson, J., Axford, I., Barnes, T., Buckeridge, J., Carrington, G., Forster, R., Huckerby, J., Idriss, H., Jones, G., Krumdieck, S., Maxwell, I., Packer, M., Salinger, J., Saunders, C., Sims, R., White, P., Weston, J., 2006. 2020: Energy opportunities, Report of the Energy Panel. The Royal Society of New Zealand, Wellington, pp. 1–68.
Weissman, J.C., Goebel, R.P., 1987. Design and analysis of pond systems for the purpose of producing fuels, Report to the Solar Energy Research Institute, Golden, Colorado, pp. SERI/STR-231-2840.
Whitaker, R.H., Likens, G.E., 1975. The biosphere and man. In: Lieth, H., Whitaker, R.H. (Eds.), Primary Productivity of the Biosphere. Springer, Berlin, pp. 305–328.
Williams, L.A., Foo, E.L., Foo, A.S., Kuhn, I., Heden, C.G., 1978. Solar bioconversion systems based on algal glycerol production. Biotechnol. Bioeng. Symp. 8, 115–130.
Woodward, J., Mattingly, S.M., Danson, M., Hough, D., Ward, N., Adams, M., 1996. In vitro hydrogen production by glucose dehydrogenase and hydrogenase. Nat. Biotechnol. 14, 872–874.
Woodward, J., Orr, M., Cordray, K., Greenbaum, E., 2000. Enzymatic production of biohydrogen. Nature 405, 1014–1015.
Yanagi, M., Yoshitomo, W., Saiki, H., 1995. CO_2 fixation by *Chlorella sp*. HA-1 and its utilization. Energy Convers. Manage. 36, 713–716.
Yu, T.-H., 2008. World Oilseeds and Products, FAPRI 2008 Agricultural Outlook. Food and Agricultural Policy Research Institute (FAPRI).
Zeiler, K.G., Heacox, D.A., Toon, S.T., Kadam, K.L., Brown, L.M., 1995. The use of microagae for assimilation and utilization of carbon dioxide from fossil fuel-fired power plant flue gas. Energy Convers. Manage. 36, 707–712.