

CO₂ MITIGATION AND RENEWABLE OIL FROM PHOTOSYNTHETIC MICROBES: A NEW APPRAISAL

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Abstract. The only major strategy now being seriously considered for biological mitigation of atmospheric CO₂ relies entirely on terrestrial plants. Photosynthetic microbes were the focus of similar consideration in the 1990s. However, two major government-sponsored research programs in Japan and the USA concluded that the requisite technology was not feasible, and those programs were terminated after investing US\$117 million and US\$25 million, respectively. We report here on the results of a privately funded US\$20 million program that has engineered, built, and successfully operated a commercial-scale (2 ha), modular, production system for photosynthetic microbes. The production system couples photobioreactors with open ponds in a two-stage process – a combination that was suggested, but never attempted – and has operated continuously for several years to produce *Haematococcus pluvialis*. The annually averaged rate of achieved microbial oil production from *H. pluvialis* is equivalent to >420 GJ ha⁻¹ yr⁻¹, which exceeds the most optimistic estimates of biofuel production from plantations of terrestrial “energy crops.” The maximum production rate achieved to date is equivalent to 1014 GJ ha⁻¹ yr⁻¹. We present evidence to demonstrate that a rate of 3200 GJ ha⁻¹ yr⁻¹ is feasible using species with known performance characteristics under conditions that prevail in the existing production system. At this rate, it is possible to replace reliance on current fossil fuel usage equivalent to ~300 EJ yr⁻¹ – and eliminate fossil fuel emissions of CO₂ of ~6.5 GtC yr⁻¹ – using only 7.3% of the surplus arable land projected to be available by 2050. By comparison, most projections of biofuels production from terrestrial energy crops would require in excess of 80% of surplus arable land. Oil production cost is estimated at \$84/bbl, assuming no improvements in current technology. We suggest enhancements that could reduce cost to \$50/bbl or less.

Keywords: biodiesel, bioenergy, bioengineering, biofuels, microalgae, microbes, mitigation, photobioreactor, photosynthesis

1. Introduction

Significant recent changes in global climate have launched a massive international research effort to observe, understand, and predict climate (IPCC 2001a). Unprecedented anthropogenic emissions of greenhouse gases are probably the cause of recent climate change. All indications are that, if left unchecked, accelerating greenhouse gas (GHG) emissions during this century will likely lead to even more dramatic changes in the Earth’s climate system. Mitigation strategies have therefore

become a focus of intensive research, and the principal goal of international environmental policy (UNFCCC 1997).

In 1996, the IPCC began a major initiative to understand future GHG emissions in the absence of any specific policies to mitigate climate change; this resulted in the Special Report on Emissions Scenarios (SRES; Nakicenovic et al. 2000) that established six basic groups of scenarios. Modelers from around the world were then invited to quantify the impact of a broad variety of mitigation scenarios, resulting in a comparison of multiple “post-SRES mitigation scenarios” (Morita et al. 2000). The post-SRES models were all focused on stabilizing atmospheric CO₂ at a specified concentration.

We highlight two important features of the post-SRES models. First, the lowest CO₂ stabilization target considered by most of the mitigation models is 450 ppm. This is more than 80 ppm greater than the current concentration, a value which has very likely not been exceeded in the past 20 million years (Pearson and Palmer 2000). Second, the modeled mitigation strategies employ a wide variety of options, ranging from those that result in net negative costs, such as improved, energy-conserving design of buildings and appliances, to potentially costly and technologically challenging replacement of fossil fuel by a variety of energy sources.

Biological strategies are generally considered to be just one element of mitigation. For example, the Second Assessment Report (IPCC 1995) concluded that biological mitigation options could offset 10–20% of projected fossil fuel emissions by 2050. A recent review of 17 studies that evaluate the contribution of biomass to the future world energy supply underscores the point that renewable energy from biomass is usually considered to be only one of many components of an overall mitigation strategy, most of which include continuing reliance on fossil fuels to some degree (Berndes et al. 2003). Studies that specify a complete phasing out of fossil fuels by 2100 (e.g. Lazarus et al. 1993; Yamamoto et al. 1999) explicitly include biofuels as one energy source, but assume these will be derived entirely from terrestrial plants and exclude any consideration of photosynthetic microbes.

Research and technology development on the production of biofuels from photosynthetic microbes began intensively in the 1980s and continues today. Most of the published evaluations suggest that microbial-based biofuel processes may not be achievable with current technology. One of the most intensive publicly funded research programs to study the feasibility of microbial-based biofuels, the Aquatic Species Program (ASP) of the U.S. National Renewable Energy Laboratory (NREL), invested more than US\$25 million over a period of 20 years and terminated in the late 1990s; the ASP concluded that economical production of biofuels from photosynthetic microbes was not likely to be feasible (Sheehan et al. 1998). The coauthors of this paper are cofounders of a marine biotechnology company that, in the 4 years from 1998 to 2001, invested US\$20 million to conduct research on the same topic. Our findings, based on large-scale pilot operations, came to a different conclusion. We report these findings here for the first time.

The published literature on mitigation strategies tends to dismiss technologies that are not currently viable (e.g. Rubin et al. 1992) or, if additional research and development is required to render them viable, the costs and benefits are more often incorporated as general terms in a model (e.g. Edmonds 2004) rather than explicitly identified.

We take the view in this paper that the production of biofuels from photosynthetic microbes may be the most promising of all biological mitigation strategies. First, we postulate a target of fossil-fuel independence by 2020 and, for context, summarize some of its projected consequences. Second, we review the advantages of biofuel production from photosynthetic microbes; we discuss the scientific and technical progress made to date, including recent advances made in industry that have not been reported in the scientific literature. Third, we estimate economic feasibility of the process.

2. A Fossil Fuel Free Future and the Upper Limit of Bioenergy

The current consensus of the scientific community is that atmospheric CO₂ concentrations will continue to rise in direct proportion to fossil fuel use, with significant consequences for global climate; alternatively, reductions in fossil fuel use will diminish the risk of many negative outcomes of GHG-induced climate change. Clearly, the optimal mitigation scenario for the future involves a transition to renewable energy.

The projected global demand for energy sets an upper limit on the amount of CO₂ that would be emitted into the atmosphere by continued reliance on fossil fuels, and therefore defines the mitigation target. Our goal is to critically examine the upper limit of bioenergy production. Our strategy of inquiry is as follows. First, we suggest a scenario in which all electric power production from fossil fuels, which is otherwise projected to account for 40% of global-fossil fuel CO₂ emissions in 2020 (Table I), is eliminated by 2020. Second, we propose a scenario in which all remaining uses of fossil fuel are converted to bioenergy. We then compare the prospects for production of bioenergy from terrestrial versus aquatic plants.

2.1. GLOBAL ENERGY DEMAND AND CARBON EMISSIONS

Total fossil fuel energy demand in 2000 of 323 EJ yr⁻¹ is projected to grow to 403 EJ yr⁻¹ in 2010, and to 488 EJ yr⁻¹ in 2020 (Table I). Nonfossil energy sources of electricity, dominated by hydroelectric power, nuclear, and renewables, add an additional 38, 43, and 48 EJ yr⁻¹ (IEA 1998), bringing total energy demand to 361, 446, and 553 EJ yr⁻¹, respectively.

Total atmospheric emissions from fossil fuels are projected to increase from 6.79 GtC yr⁻¹ in 2000 to 8.35 GtC yr⁻¹ in 2010 and 9.97 GtC yr⁻¹ in 2020 (Table I).

TABLE I
Projected global energy demand and CO₂ emissions, 2000 to 2020

Energy source and use	Demand (EJ/year)			Emissions (GtC/year)		
	2000	2010	2020	2000	2010	2020
Oil – electricity ^a	14	15	18	0.27	0.31	0.35
Oil – transport ^b	69	97	119	1.60	2.16	2.65
Oil – other ^c	64	71	75	1.25	1.38	1.47
Total oil ^d	147	182	212	3.12	3.85	4.47
Coal – electricity ^a	65	85	106	1.68	2.19	2.73
Coal – other ^c	27	22	17	0.70	0.57	0.43
Total coal ^d	92	107	123	2.38	2.76	3.16
Natural gas – electricity ^a	29	43	62	0.44	0.66	0.95
Natural gas – other ^f	55	71	91	0.84	1.09	1.39
Total natural gas ^g	84	114	153	1.29	1.74	2.34
Total fossil fuels	323	403	488	6.79	8.35	9.97
Fossil electricity	108	143	186	2.39	3.16	4.03
Non-fossil electricity ^a	38	43	45			
SRES Scenario A1T ^h				6.90	8.33	10.00
Total energy demand	361	446	533			

^aDemand and emissions from IEA (1998).

^bDemand from EIA (1999); emissions from WEC (1998).

^cDemand calculated by difference; emissions assume conversion for electricity, i.e. 19.5 MtC/EJ.

^dDemand from EIA (2003a).

^eDemand calculated by difference; emissions assume conversion for electricity, i.e. 25.8 MtC/EJ.

^fDemand calculated by difference; emissions assume conversion for electricity, i.e. 15.3 MtC/EJ.

^gDemand from EIA (2003b).

^hIPCC (2001c, Appendix II).

The emissions estimates in Table I agree well with estimates from SRES scenario A1T of 6.90, 8.33, and 10.00 GtC yr⁻¹, although they are based on independent projections, many of which postdate the IPCC analysis.

2.2. ELECTRICITY PRODUCTION WITH ZERO CARBON EMISSIONS BY 2020

The International Energy Agency has projected that global production of electricity will continue to depend significantly on fossil fuels, with a total requirement in excess of 27,000 TWh in 2020 (IEA 1998 and Table II). In the IEA scenario, fossil fuels would account for about 75% of total electricity production. The bulk of

TABLE II
Two scenarios of projected electricity production in 2020

Energy source	IEA scenario electricity generation (TWh)	IEA scenario percent of total (%)	Non-fossil scenario electricity generation (TWh)	Non-fossil scenario percent of total (%)
Oil	1,941	7.1	0	0
Coal	10,296	37.7	0	0
Gas	8,243	30.2	0	0
Total fossil fuels	20,480	75.0	0	0
Nuclear	2,317	8.4	2,317	8.5
Hydro	4,096	15.0	8,708	32.0
Solar			49	0.2
Wind			16,144	59.3
Renewables	433 ^a	1.6		
Total non-fossil	6,846	25.0	27,219	100.0
Total	27,326	100.0	27,219	100.0

The International Energy Agency scenario is based on IEA (1998). The non-fossil fuel scenario is discussed in the text.

^aThe IEA estimate for “renewables” includes solar, wind and biomass sources of energy.

the remainder would be made up by hydroelectric power (15%) and nuclear power (8%). Renewables, including wind, solar, and bioenergy, are accorded a minor share of the total (2%).

We envision an alternate scenario in which zero-emission technologies entirely replace fossil fuel technology for electricity production by 2020 (Table II). We suggest that nuclear power production will remain stable at about 2317 TWh (IEA 1998). Economically viable hydroelectric production could be expanded by 2020 to between 6964 TWh (World Atlas 1999) and 8708 TWh (International Water Power and Dam Construction 1997). Photovoltaic power production would reach only about 49 TWh (Table II), even if it continues to expand at a growth rate of 25% annually (Frantzis 2003) from its current base of about 1.1 TWh in 2000 (Darmstadter 2003). Installed wind power capacity in 2003 produced 98 TWh, a 26% increase over the previous year in an industry that has experienced sustained global growth of 25% per year for more than a decade, and growth in Europe of 35% per year for the past 5 years (EWEA 2004). Wind power production would reach 16,144 TWh by 2020 (Table II) based on the current growth rate in Europe. Together, these non-fossil sources of electricity generation could satisfy the projected demand of more than 27,000 TWh. We do not argue strongly for any details of this scenario – we merely point out that electricity from sources other than fossil fuels is conceivable by 2020.

2.3. REPLACEMENT OF REMAINING FOSSIL FUELS WITH BIOENERGY

The total energy demand from fossil fuels in 2020 is projected to be 488 EJ yr⁻¹ (Table I). We have suggested above that electricity generation (186 EJ yr⁻¹) can be entirely replaced with renewables. This leaves 302 EJ yr⁻¹, of which 39% (119 EJ yr⁻¹) consists of oil-based liquid fuels for transportation, and the remaining 61% (183 EJ yr⁻¹) is required for other uses such as home heating and manufacturing.

Here we examine the potential for bioenergy to provide approximately 300 EJ yr⁻¹ by 2020. Our zero-order analysis is simple and straightforward. Terrestrial ecosystems are not sufficiently productive to provide this amount of bioenergy on the amount of land that is available. The total land potentially available for crop production is 2.6 Gha, or about 19.5% of the Earth's land surface; of this amount, approximately half (1.31 Gha) is estimated to be in cultivation by 2050, leaving only 1.28 Gha for the potential production of biomass energy crops (IPCC 2001b, Table 3.31).

Table III summarizes the results of 11 scenarios from nine modeling studies conducted over the past decade aimed at estimating global bioenergy production and associated land requirements. None of the model results generated as much as 300 EJ yr⁻¹. Most of the model results are projections for the period 2025 to 2030, except for two models projected to 2050 (Nakicenovic et al. 1998; Sørensen 1999). All models incorporated yield data that fall within the range of observations for a variety of natural ecosystems (Amthor and Huston 1998) or managed energy plantations (Paustian et al. 1998), most of which fall below 400 GJ ha⁻¹ yr⁻¹. Many of the models took into account details on the type of land available, its suitability for certain crops and, in some cases, competition for non-energy land use (e.g. Nakicenovic et al. 1998). All but one of the models (Fischer and Schrattenholzer 2001) proposes less than 1.0 Gha be devoted to bioenergy production. Based on assumed areal productivities, only one of the 11 scenarios delivers 300 EJ yr⁻¹ on an area less than 1.0 Gha (Lazarus et al. 1993), and 8 of the 11 scenarios would require more than the projected available amount of 1.28 Gha to deliver 300 EJ yr⁻¹.

Bioenergy from terrestrial plants already contributes >45 EJ yr⁻¹ to the global energy supply (Fischer and Schrattenholzer 2001). No doubt this supply will increase, especially for resources such as crop residues that continue to be supplied and for which energy generating capacity already exists. However, to attain more than 100 EJ yr⁻¹ from terrestrial sources of bioenergy is likely to require that most of the Earth's remaining arable land be devoted to plantations of energy crops (Table III). This approach appears, on its face, to be untenable.

We suggest a re-examination of the potential for bioenergy from photosynthetic microbes. We will show in the remainder of this paper that current production technology has already delivered an annually averaged yield of more than 400 GJ ha⁻¹ yr⁻¹. We will further show that yields of more than 3000 GJ ha⁻¹ yr⁻¹

TABLE III
Observed and modeled productivity of natural terrestrial ecosystems and managed crops compared to observed and projected productivity of photosynthetic microbes

System or model	Biomass production (ODT/ha/yr)	Carbon production (TC/ha/yr)	Energy production (GJ/ha/yr)	Land area available (Gha)	Energy produced (EJ/yr)	Land required for 300 EJ/yr (Gha)	Reference
Ecosystems							
Temperate grassland	7.8	3.50	156				Amthor and Huston (1998)
Temperate forest	14.9	6.70	298				Amthor and Huston (1998)
Tropical forest	20.6	9.25	411				Amthor and Huston (1998)
Managed systems							
Total cropland, 2050				1.31			IPCC (2001b, table 3.31)
Cultivated crops	9.4	4.25	189				Amthor and Huston (1998)
Temperate energy crops	15.6	7.00	311	0.05	15	0.96	Paustian et al. (1998)
Tropical energy crops	20.0	9.00	400	0.05	19	0.75	Paustian et al. (1998)
Energy cropland, 2050	15.0	6.75	300	1.28	396	0.97	IPCC (2001b, Table 3.31)
Model systems							
Cropland + rangeland	3.0	1.35	54	0.75	41	5.56	Sørensen (1999)
Grassland	3.8	1.71	76	2.17	165	3.95	Fischer and Schratzenholzer (2001)
Various, demand-driven	4.6	2.07	92	0.19	18	3.26	Leemans et al. (1996)
Forest + cropland	6.0	2.70	120	0.87	104	2.50	Swisher and Wilson (1993)
Plantations	8.4	3.78	168	0.61	102	1.79	Nakicenovic et al. (1998)
Crop + degraded land	10.2	4.59	204	0.08	17	1.47	Williams (1995)
Crop + degraded land	10.8	4.86	216	0.37	80	1.39	Johansson et al. (1993)
Plantations	11.7	5.27	234	0.38	90	1.28	Lazarus et al. (1993)
Plantations	12.6	5.67	252	0.39	98	1.19	Nakicenovic et al. (1998)
Plantations	15.0	6.75	300	0.89	267	1.00	Hall et al. (1993)
Plantations	23.4	10.53	468	0.16	74	0.64	Lazarus et al. (1993)

(Continued on next page)

TABLE III
(Continued)

System or model	Biomass production (ODT/ha/yr)	Carbon production (TC/ha/yr)	Energy production (GJ/ha/yr)	Land area available (Gha)	Energy produced (EJ/yr)	Land required for 300 EJ/yr (Gha)	Reference
Photosynthetic microbes							
<i>Haematococcus pluvialis</i>	38.2 ^a	17.2	422 ^b			0.71	This paper – average achieved
<i>Haematococcus pluvialis</i>	91.8 ^a	41.3	1014 ^b			0.30	This paper – maximum achieved
Other species	207 ^c	93.1	3201 ^c			0.09	This paper – projected

Original sources provided biomass production unless otherwise noted, from which carbon production was calculated assuming that phytomass is 45% C (Amthor and Huston 1998). Energy production per hectare was calculated assuming 20GJ/ODT (IPCC 2001b, Table 3.31) unless otherwise noted. "Land area available" is the amount estimated by the referenced study, and "Energy Produced" is the amount of bioenergy produced on the available land. The land required to produce 300 EJ/year is estimated from observed or modeled productivity.

^aBased on industrial-scale cultivations of *Haematococcus pluvialis* over a 1-year period ($n = 182$).

^bAssumes that only the oil fraction is used for biofuel production; see calculations in Section 4.3.3.

^cSee Table VI and calculations in Section 4.2.

are possible (Table III), and will suggest specific research that could lead to even greater yields.

3. Bioenergy from Photosynthetic Microbes

Heterotrophic microbes form the basis of vast global enterprises that encompass commodities, fine chemicals, and pharmaceuticals with annual revenues in hundreds of billions of dollars. The industrial-scale cultivation processes upon which these businesses depend has been the focus of incalculable investments in research and development, and those investments have accelerated since the Industrial Revolution as markets have grown.

Photosynthetic microbes, by contrast, have received scant attention. The productivity of photosynthetic microbes in nature, on an areal basis, exceeds that of terrestrial plants by approximately one order of magnitude (Longhurst et al. 1995). The biodiversity of photosynthetic microbes is enormous – estimated at more than 100,000 species (Sheehan et al. 1998) – and yet most of it remains biochemically and metabolically unexplored. To date, only four species have been cultivated at industrial scale.

It is worthwhile to review in some detail the history of research and development on bioproducts from photosynthetic microbes. Agriculture began more than 5000 years ago. Industrial microbiology was a global business by the mid-twentieth century. By contrast, the first attempts at large-scale cultivation of photosynthetic microbes began only 50 years ago. Their potential for bioenergy production was not recognized until the 1970s, and the resources devoted to this potential have been trivial by comparison to those lavished upon alternatives. Major advances in the biochemistry of photosynthetic microbes were made in the 1980s and 1990s. Models of bioenergy production based on laboratory results showed great promise, and significant funding flowed into further studies, especially in Japan and the USA. However, until recently all attempts to scale up results from the bench failed. Here we report on the first process operated sustainably at hectare scale.

3.1. CULTIVATION OF PHOTOSYNTHETIC MICROBES

The first photosynthetic microbe to be isolated and grown in pure culture was the freshwater microalga, *Chlorella vulgaris* (Beyerinck 1890). Over the next several decades, hundreds of species were gathered and maintained in very small quantities to form permanent culture collections (e.g. Pringsheim 1928), but very few species were cultivated in volumes of 50 ml or more (Allen and Nelson 1910; Warburg 1919).

The chemical composition of microalgae could not even be studied until the 1930s, when a new technique for “large-scale cultivation” (27 l) made it possible to collect sufficiently large samples for analysis (Ketchum and Redfield 1938). The

discovery of hydrogen production by microalgae came very soon thereafter (Gaffron and Rubin 1942). The first laboratory “photobioreactors,” developed in the 1940s (Myers and Clark 1944; Ketchum et al. 1949), made it possible to control the critical variables of light, nutrients, and temperature. Scientists at the Division of Plant Biology of the Carnegie Institution quickly adopted the use of photobioreactors in their studies of photosynthesis. Their most important finding was that the chemical composition of *Chlorella* could be dramatically altered by cultivation conditions, from 8.7% protein and 86% lipid (oil) to 58% protein and 4.5% lipid (Spoehr and Milner 1949). The authors were most interested in the potential to produce low-cost protein, and advocated “a revolutionary change in methods of food production” (Spoehr and Milner 1948).

The Carnegie Institution supported substantial investigations on large-scale culture of microalgae that began in 1951 (Burlew 1953). The largest cultivation system – a 4500-l, temperature-controlled, tubular photobioreactor – operated for 3 months. This was to be the largest closed-system photobioreactor to be operated for the next 48 years.

The concept of industrial-scale food production from microalgae began to catch on around the world. However, in contravention to basic principles of industrial microbiology, and with an apparent view to keeping costs down, the universal approach was to develop open-air, non-sterile cultivation systems. “Open ponds” are shallow (generally <15 cm) recirculating raceways, exposed to the atmosphere, in which the culture medium is recirculated by a paddlewheel device. The first open ponds were built in Germany at a scale of 1200 l (Gummert et al. 1953), followed in Japan by systems that escalated in size from 3000 l in 1953 to 16,000 l in 1957 and 60,000 l the following year (Kanazawa et al. 1958). In the USA, open pond systems up to 1,000,000 l were developed to treat wastewater (Oswald 1973). Pursuit of inexpensive protein production in open ponds proliferated to Czechoslovakia (1960), Poland (1966), Rumania (1968) and, in the 1970s, to countries on all continents but Antarctica (Goldman 1979).

Hundreds of species were tested in the laboratory, and attempts were made to grow numerous species in open ponds during the 1960s and 1970s (Goldman 1979, Table 1). However, after an additional 30 years of continued attempts to cultivate other species, sustained open pond production proved feasible for only three taxa: *Spirulina platensis*, *Dunaliella salina* and *Chlorella*, in all cases because contamination by other species can be avoided. For example, the cyanobacterium, *S. platensis*, grows best in highly alkaline media with a pH of up to 10 (Zarrouk 1966; Jimenez et al. 2003); *D. salina* is the most salt-tolerant eukaryotic alga known (Brown and Borowitzka 1979), and produces its maximum intracellular concentrations of commercially valuable β -carotene at salinities up to ten-fold greater than seawater (Borowitzka et al. 1984).

Open ponds can be an important and cost-effective component of large-scale cultivation technology, and optimal design parameters have been known for many years. The elongated “raceway-type” of open pond, using paddlewheels for

recirculation and mixing, was developed in the 1950s by the Kohlenbiologische Forschungsstation in Dortmund, Germany, and has changed little since (Becker 1978; Goldman 1979). More than 20 years later, it was declared that “Mixed systems of horizontal channels with a single loop paddlewheel are the most successful for large scale industrial production” (Laing and Ayala 1990). The optimal depth of such ponds is generally agreed to be approximately 10–20 cm (Dugan 1980; El-Fouly et al. 1984; Jassby 1988); the critical parameter here is light penetration, which clearly is dependent on cell concentration. Engineering studies suggest that maximum feasible dimensions are about 3000 m in length and 60 m in width (Oswald and Golueke 1960), about 35 times larger than the largest ponds ever constructed (Jassby 1988). The suggested limits to parameters that emerged from several decades of study – shape, method of circulation, depth, length and width – are crucial to determining the economics of open pond culture systems.

3.2. BIOFUELS FROM PHOTOSYNTHETIC MICROBES: THE AQUATIC SPECIES PROGRAM AND RELATED RESEARCH AND DEVELOPMENT

From 1978 to 1996, the US Department of Energy invested more than \$25 million in a program to develop renewable transportation fuels from microalgae (Sheehan et al. 1998). The Aquatic Species Program (ASP) focused on two apparently convergent approaches – first, to collect and identify photosynthetic microbes that produce high concentrations of oil and then to determine the cultivation conditions under which they do so; and, second, to design and demonstrate the operation of large-scale cultivation systems for the production of biofuel feedstock, using species that had been developed in the laboratory. The ASP did not achieve its main goal, concluding in its final report that “even with aggressive assumptions about biological productivity, we project costs for biodiesel which are two times higher than current petroleum diesel fuel costs” (Sheehan et al. 1998, p. ii). It is understandable, therefore, that the IPCC has focused on the potential for biofuel production from other sources of biomass where the economics appear more favorable.

The ASP made significant scientific progress in its laboratory studies. The large-scale cultivation program, however, did not deliver the productivities estimated by the extrapolation of laboratory results. Here, we summarize some of the principal findings of the program.

The ASP built on many basic findings and concepts that already had been made or proposed. Photosynthetic microbes are a major contributor to geological formations of hydrocarbons that are now the source of fossil fuels (Dyner 2003). Microalgae had long been recognized to be more than one order of magnitude more productive per unit area than terrestrial plants (Warburg 1919). It was also well known that oil content was a function of the culture conditions, and in particular that oil production was stimulated by nutrient deprivation (Spoehr and Milner 1949). Scientists at the University of Göttingen had begun experimenting in 1939 with the possibility of

oil production from a diatom (Johnston 1976), and it was not long after that other German scientists suggested that microalgae could provide a means for “biological utilization of huge quantities of carbon dioxide from waste gases available in the industrial district of the Ruhr” (Gummert et al. 1953).

Laboratory studies under the ASP carefully quantified earlier findings. A culture collection of more than 3000 strains was amassed (Barclay et al. 1986). Research on many strains demonstrated that, in general, nitrogen-sufficiency promoted high growth rates and low oil content, whereas nitrogen-deficiency reduced growth rates and resulted in high oil content (Tornabene et al. 1983; Lewin 1985). Silicon deficiency in diatoms yielded similar results (Tadros and Johansen 1988); oil content accumulated to approximately 60% in a variety of culture trials. By the early 1990s, it was concluded that “the desirable traits for biodiesel production (high productivity and high lipid content) were found to be mutually exclusive. . . [and] therefore it was decided to use mutagenesis or genetic engineering to manipulate the algal biosynthetic pathways to produce algal strains with constitutively high lipid levels” (Sheehan et al. 1998, p. 113). This research led to the first successful genetic transformation of a diatom (Dunahay et al. 1995), but its subsequent application did not result in an increase in oil accumulation.

The large-scale cultivation part of the program, meanwhile, was going in another direction. The ASP solicited two independent designs for a test facility, one employing open ponds and the other proposing to use tubular photobioreactors. The open pond proposal was accepted, and the test facility was built in Roswell, New Mexico in 1988. The facility consisted of two “large” (1000 m²) ponds of the paddlewheel raceway variety. Operating results were similar to those obtained from open pond cultures in the three preceding decades. Several promising strains of algae from the ASP collection (*Cyclotella cryptica*, *Tetraselmis suecica*, and *Monoraphidium minutum*) were inoculated in the ponds (Weissman and Tillett 1992). However, as in prior experience, monospecific cultures could not be dependably maintained for more than a few weeks or months (cf. Goldman 1979, Table 1). The ASP final report referred to this result as an “uncertainty [with] the nature of species control achieved” (Sheehan et al. 1998, p. 198).

The results of modeling studies under the ASP provide some indication of the economics of biodiesel production (Table IV). The projected cost of microalgae oil in these scenarios ranged from \$39 to \$127 per bbl. The cost of biodiesel production from feedstock is a minor component (Ma and Hanna 1999). The production model assumes an oil content at 40% of dry weight and an average daily production rate ranging from 18.5 to 60 g m⁻² d⁻¹. The values for these parameters are within the range of what has been observed. Sustained productivity of >20 g m⁻² d⁻¹ has been frequently observed in open ponds, with brief periods attaining up to 70 g m⁻² d⁻¹ (Goldman 1979; Laws et al. 1986). Oil content can be much higher than 40% – up to 59% in *Stichococcus* (Lewin 1985), and 86% in *Botryococcus braunii* (Wolf 1983) – but such high values occur only after the cells have been stressed by nutrient deficiency and may no longer be dividing (Wolf et al. 1985).

TABLE IV

Productivity models and economic analysis for biofuels production from microalgae, based on two scenarios from each of the two models

Variable	A	B	C	D
Algae production (g m ⁻² d ⁻¹)	18.5	18.5	30.0	60.0
Algae production (MT ha ⁻¹ yr ⁻¹)	67.5	67.5	109.5	219.0
Oil content (%)	40	40	40	40
Oil production (T ha ⁻¹ yr ⁻¹) ^a	27.0	27.0	43.8	87.6
Oil production (bbl ha ⁻¹ yr ⁻¹)	160	160	380	760
Annual operating cost (\$/ha) ^b	\$20,385	\$9,830	\$26,293	\$29,370
Production cost (\$/bbl)	\$127	\$61	\$69	\$39

Scenarios A and B assume only differences in costs (Benemann et al. 1982); scenarios C and D assume differences in both productivity and costs: (Benemann and Oswald 1996). Algae production is expressed in units of dry weight. All values are as originally reported by the authors; no corrections were made for inflation.

^aScenarios A and B assume 168.8 kg per bbl; scenarios C and D assume 115 kg per bbl.

^bIncludes depreciated capital costs.

The models in Table IV would require oil production rates in the range of 7.4 to 24 g m⁻² d⁻¹; however, oil production rates in cultures grown under nutrient deficiency are generally <5 g m⁻² d⁻¹ (Thomas et al. 1984a,b).

The major conclusion of the ASP with regard to their economic models was that “microalgae production for fuels is currently not limited by engineering designs, but by the many microalgae cultivation issues, from species control in large outdoor systems to harvesting and lipid [oil] accumulation to overall productivity” (Sheehan et al. 1998, p. 247). The ASP also concluded that “. . . [photobioreactors] are not likely to be an essential or crucial component of large-scale, low-cost microalgae culture processes for energy production” (Sheehan et al. 1998, p. 262).

Japan committed about US\$117 million (Masutani and Nakamura 1998) to conduct research on microalgal CO₂ utilization in the 1990s in a program entitled Research Institute of Innovative Technology for the Earth (RITE), funded by the Ministry of International Trade and Industry (MITI) through the New Energy Development Organization (NEDO). Like the ASP, the program focused on both species collection and characterization (e.g. Nishikawa et al. 1992; Murakami and Ikenouchi 1997) and development of cultivation technology. Unlike the ASP, RITE chose to pursue the development of photobioreactors rather than open ponds. However, none of the photobioreactors developed in the laboratory (Matsunaga et al. 1991; Burgess et al. 1993; Usui and Ikenouchi 1997) appear to have been attempted at industrial scale. RITE discontinued its research on biological fixation of CO₂ in 1999, and has since turned its attention entirely to geological sequestration (RITE 2004).

3.3. PHOTOBIOREACTOR TECHNOLOGY FOR THE INDUSTRIAL SCALE CULTIVATION OF MICROALGAE

In the late 1980s we became interested in developing industrial-scale photobioreactors for the production of valuable bioproducts from microalgae. At that time, after more than three decades of worldwide research and development on open pond cultivation systems, commercial production had proven feasible for only three genera of microalgae – *Chlorella*, *Spirulina* and *Dunaliella* – despite a monumental investment of resources and numerous attempts to cultivate many species (Goldman 1979; Sheehan et al. 1998). Contamination was clearly inevitable in long-term open pond cultures of all microalgae but those that could tolerate most unusual environmental conditions. The only solution was to enclose the microbial culture and to provide the means to control fundamental variables of pH, temperature, nutrients and light.

The company we founded, Aquasearch, sought and received several patents for our photobioreactor-based production process (Huntley et al. 1996, 1997, 1999). In the period from mid-1997 through mid-2001 we invested US\$20 million in the construction and operation of a 2 ha production facility at the Hawaii Ocean Science and Technology Park at Keahole Point, Hawaii island. The facility consisted of 25,000-l “production” photobioreactors and 50,000 l open ponds; it had total capacity of >600,000 l, equally divided between photobioreactors and ponds.

The project was a success, measured by the fact that in 1999 – only 2 years after beginning construction – we brought to market the first new commercial product from microalgae since the advent of cultured *Spirulina* in 1982. The product, an extract of the valuable carotenoid pigment, astaxanthin, is derived from *Haematococcus pluvialis*, a species that had long been recognized as having great commercial potential. Attempts to cultivate *H. pluvialis* at large scale in open ponds had met with failure for many years previous (Bubrick 1991).

3.3.1. Industrial-Scale Cultivation of *Haematococcus Pluvialis* for Production of Biofuel Feedstock from Microalgae

Haematococcus pluvialis produces oil under the same conditions that it produces astaxanthin. Under nutrient-sufficient conditions, the cells are highly productive, but contain relatively small amounts of either product. Under conditions of nutrient depletion, cell division and productivity are greatly reduced, and are accompanied by enhanced biosynthesis. After less than 2 days, astaxanthin content increases, and oil content reaches 25% of dry weight (Zhekisheva et al. 2002).

From a bioengineering perspective the conceptual solution to maximizing the production of both oil and astaxanthin from *H. pluvialis* is to adopt a two-stage process (Figure 1). The first stage requires maintaining constant conditions that favor continuous cell division, and prevent contamination of the culture by other organisms. Clearly, such conditions are best maintained in a photobioreactor. The goal of the second stage is expose the cells to nutrient deprivation and other environmental

CO₂ MITIGATION AND RENEWABLE OIL FROM PHOTOSYNTHETIC MICROBES

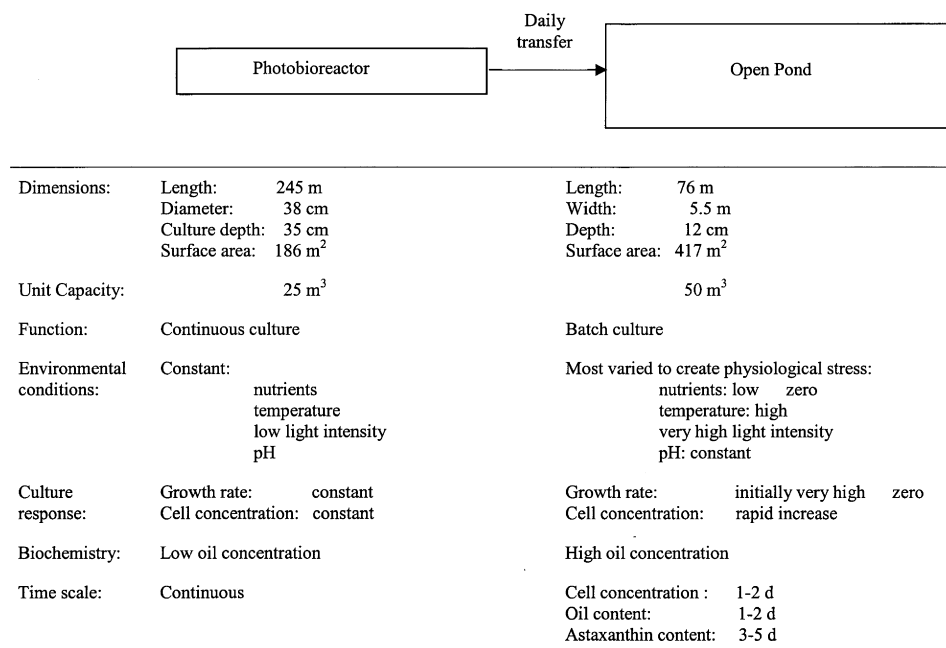


Figure 1. General specifications of the Aquasearch-coupled production system for photosynthetic microbes.

stresses that lead, as rapidly as possible, to synthesis of the product of interest – whether astaxanthin or oil. Environmental stresses that stimulate oil production can be applied rapidly by transferring culture from the photobioreactor to an open pond.

3.3.2. Technology Description and Method of Operation

Here we summarize key features of the cultivation system and its methods of operation, most of which have been reported elsewhere in more detail (Olaizola 2000, 2003).

3.3.2.1. *Photobioreactor*. The main body of the production photobioreactor is a long tubular plastic chamber (38-cm diameter) that is laid on an impermeable surface and doubled back on itself several times, creating a series of four parallel tubes that are connected through a single “end assembly.” The entire reactor chamber is partially immersed in a water bath that provides temperature control. The end assembly, outside the water bath, incorporates a pump or airlift that generates a recirculating turbulent flow of sufficient velocity to keep cells in suspension, generally a Reynolds number (Re) in the range of 2000 to 20,000. The “end assembly” also contains pH and temperature sensors and automated plumbing fixtures connecting the photobioreactor to the nutrient media supply and to the open ponds. All operations are performed automatically via proprietary industrial process-control software.

The photobioreactor is exposed to full sunlight, which at Keahole Point attains a maximum midday intensity of $>2200 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ in summer (Olaizola 2003). We maintained temperature in the range of 16° to 18°C ; higher temperatures trigger the addition of cold seawater to the water bath until the desired reduction in temperature is achieved. We maintained pH in the range of 7.3 to 7.8 by the addition of CO_2 , again until a desired endpoint is achieved. The culture medium we used is a modified Bold's Basal medium (Bischoff and Bold 1963).

The scale-up system began in the laboratory with a series of 20-l carboy cultures. These provided a total of 180 l of inoculum to initiate a small outdoor photobioreactor (1000 l). After one more step (5000 l), we scaled up to the production photobioreactor. The entire scale-up process was completed in 2 weeks. We routinely maintained production photobioreactors at steady state for 90 days by harvesting daily a fraction of the culture approximately equivalent to the cell division rate and transferring it directly to an open pond. After transfer, fresh culture medium was added automatically to dilute the culture and to restore nutrient concentrations.

We employed sterile technique throughout the scale-up system, using chlorination to sterilize all components of the photobioreactors prior to inoculation. Plumbing connections were likewise sterilized daily after each fractional harvest and nutrient medium addition. All sterilizing operations were conducted automatically via the process-control system.

3.3.2.2. Open Ponds. The open pond is of the common paddlewheel-driven, recirculating raceway design, fitted with a durable plastic liner. Each pond occupies an area of 417 m^2 with an average depth of 12 cm, allowing a full volume of 50 m^3 . Ponds, like photobioreactors, were exposed to full sunlight. Temperature was not controlled, and ranged from a minimum of 16°C at night to 34°C in the day. On the first day of pond operation, photobioreactor culture is transferred at dawn to a full pond of nutrient-depleted culture medium. We transferred just enough nutrients with the fresh culture to allow cell division to continue through the morning of the second day. Even before cell division ceases, oil and astaxanthin content begin to visibly increase. Under ideal conditions, as in the laboratory (Zhekisheva et al. 2002), oil content reaches 25% of dry weight by the second day after inoculation; astaxanthin content attains a maximum by the third day after inoculation, at which point the pond can be harvested, cleaned and prepared for a new production cycle.

3.3.2.3. Harvest and Processing. For *Haematococcus pluvialis*, we concentrated the cells into a slurry by gravitation, removed the excess water, then further concentrated the slurry by centrifugation. The wet biomass was then dried, and oil-soluble astaxanthin extracted by proprietary processes.

3.3.3. Operating Results

We operated the cultivation system continuously from December 1997 through September 2001, during which time we repeated the entire process >500 times.

During the first several years we significantly optimized production rates. Here we report only on the final year of operation, from September 2000 to September 2001, during which time we harvested 182 pond cultures.

Biomass in the photobioreactors was estimated from cell counts of samples collected daily at 6:00 a.m. Cells were counted using a model Z1 Coulter Counter, and abundance converted to biomass based on the average dry weight of 1×10^{-9} g per cell, which was independently determined from direct measurements of our cultures. This allowed us to accurately estimate the dry weight biomass of inoculum that was used to inoculate each pond within an hour of the biomass measurement. Biomass of pond cultures was similarly estimated during each day of operation. The specific growth rate (d^{-1}), which is exponential, is directly calculated from the increase in biomass over time.

The biomass yielded from each pond was directly measured by weighing the total wet biomass produced; we calculated dry weight biomass based on the dry:wet weight ratio measured on samples from each batch that were heated to complete dryness for 24 hr at 60 °C and weighed on a microbalance. Oil production was calculated based on the average oil content of 25% of dry weight.

We maintained an average initial photobioreactor culture concentration of 300 g (dry weight) m^{-3} at an exponential specific growth rate of 0.25 d^{-1} (Table V), which is comparable to the value of 377 g m^{-3} at a growth rate of 0.20 d^{-1} that has been determined independently for *Haematococcus pluvialis* (Fábregas et al. 2000). These relatively low growth rates are typical of light-limited conditions that arise at very high cell concentrations. Average areal productivity was 10.2 g $m^{-2} d^{-1}$, corresponding to a photosynthetic efficiency of 3.0%. The total daily production of a 25,000 l photobioreactor thus yielded 1.90 kg (dry weight) biomass.

We corrected for minor variations in photobioreactor biomass so that open ponds were routinely inoculated with 1.90 kg (dry weight) biomass, producing an initial pond concentration of 38 g (dry weight) m^{-3} (Table V). The consequent reduction in cell concentration effectively increased the light intensity per cell, and inoculation at dawn allowed for a period of photoacclimation. As a result the culture grew rapidly at a rate (1.3 d^{-1}) considered typical of optimal vegetative growth (Boussiba 2000). After the morning of the second day cell division ceased; the culture attained its maximum biomass concentration (206 g m^{-3}) at a production rate of 15.1 g $m^{-2} d^{-1}$ ($n = 182$) (Table V), equivalent to a photosynthetic efficiency of 4.4%.

The average total oil content of *H. pluvialis* after 1.3 d in the open pond culture was 25% of dry weight (cf. Zhekisheva et al. 2002). Based on the achieved production of dry weight biomass in open ponds, this yields oil production values ranging from a mean of 3.78 g (oil) $m^{-2} d^{-1}$ to a maximum of 9.09 g (oil) $m^{-2} d^{-1}$ (Figure 2). At the hectare scale of our cultivation conditions, this translates to an annual mean and maximum of 13.8 and 33.2 toe $ha^{-1} yr^{-1}$, respectively. Allowing for the additional area occupied by the photobioreactors (i.e. 186 m^2 per 417 m^2 of pond area; Figure 1), the oil production scales to a mean and maximum 9.5 and 22.9 toe $ha^{-1} yr^{-1}$, respectively. A specific gravity of biodiesel of 0.87 yields an energy

TABLE V
 Cultivation system for *Haematococcus pluvialis*: representative average results in the coupled system of continuous culture photobioreactors and batch culture open ponds for Sep. 2000 to Sep. 2001 ($n = 182$), and the maximum achieved in March 2001

Cultivation system & data set	Initial		Growth rate (d^{-1})	Growth period (d)	Final		Harvest biomass (kg)	Oil production (%)	Biomass production ($\text{g m}^{-2} \text{d}^{-1}$)
	concentration (g m^{-3})	biomass (kg)			concentration (g m^{-3})	concentration (g m^{-2})			
Photobioreactor	300	40.3	0.25	n/a^a			1.90 ^b		10.2
Open pond	38	4.6	1.29	1.3	202	24.2	10.10	25	3.78
Maximum pond: March 2001	38	4.6	1.87	1.3	432	51.8	21.6	25	9.09

All units of biomass are dry weight. Dimensions and capacities of photobioreactor and pond are identical to those shown in Figure 1. Biomass production is scaled to the surface area of the pond.

^aPhotobioreactors operate continuously.

^bBiomass harvested from the photobioreactor is used to inoculate ponds.

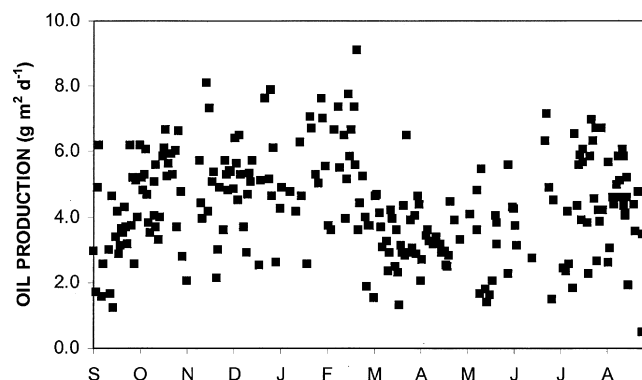


Figure 2. *Haematococcus pluvialis*: Oil production rates ($\text{g m}^{-2} \text{d}^{-1}$) in the coupled cultivation system for the period Sep. 2000 to Sep. 2001. Each data point represents the actual production from open ponds; production rates are scaled to the surface area of the pond.

density of 44.2 GJ/toe and, assuming 100% extraction efficiency, the corresponding yields of *H. pluvialis* oil are in the range of 422 to 1014 GJ ha⁻¹ yr⁻¹.

The average annual rate of dry weight biomass production from *H. pluvialis* in the ponds was 15.1 g (dry weight) m⁻² d⁻¹ (Table V). Once again correcting for the area occupied by photobioreactors, the production becomes 10.5 g (dry weight) m⁻² d⁻¹ or, on an annual basis, 38.1 tonnes (dry weight) ha⁻¹ yr⁻¹. Similarly, the maximum rate we achieved corresponds to production of 91.8 tonnes (dry weight) ha⁻¹ yr⁻¹. Allowing for a dry biomass energy content of 20 GJ tonne⁻¹ (IPCC 2001b), the total energy production of *H. pluvialis* was on average 763 GJ ha⁻¹ yr⁻¹, with a maximum value corresponding to 1836 GJ ha⁻¹ yr⁻¹.

The annual rate of energy production we achieved from photosynthetic microbes at sustained industrial scale exceeds by almost double the most optimistic model projections for terrestrial sources of biofuels (Table III). Even the annual rate of oil production alone, 422 GJ ha⁻¹ yr⁻¹, exceeds all but one of these same model projections. Extrapolating from our results to global scale, oil production from photosynthetic microbes could supplant all the non-electrical generation fossil fuel energy requirements of 300 EJ yr⁻¹ on as little as 0.30 Gha, or 23% of the Earth's available cropland (Table III). By contrast, all but one of the models of terrestrial plant energy production (Lazarus et al. 1993) would require more than all the cropland on Earth to achieve the same result.

4. Discussion

4.1. THE COUPLED PHOTOBIOREACTOR-OPEN POND CULTIVATION SYSTEM

The cultivation system described in this article represents a breakthrough in cultivation technology for photosynthetic microbes. In the half century leading up

to this research and development, industrial-scale cultivation was limited to open pond technology and was successful only for a handful of species (Goldman 1979; Borowitzka 1999). Cultivation in open ponds of photosynthetic microbes, carefully selected for their high oil content as a feedstock for biodiesel fuel, proved unsustainable (Sheehan et al. 1998).

Closed-system photobioreactors are well recognized for their excellent ability to control sterility and, in so doing, to permit continuous cultivation of a wide variety of species (Borowitzka 1996). However, their application to industrial production has been limited by small scale, generally less than 1000 l (Borowitzka 1999). Furthermore, capital costs of photobioreactors have typically been substantially greater than for open ponds, leading Sheehan et al. (1998, pp. 261–262) to conclude that “cost constraints restrict consideration of [microalgal biodiesel production systems] to the simplest possible devices, which are large unlined, open, mixed raceway ponds.”

The apparent technical conundrum: open pond technology has advanced to affordable industrial scale, but cannot provide sustainable production, while photobioreactors provide sustainable production, but have not been operated at industrial scale. The coupled photobioreactor-open pond cultivation system we developed solves the fundamental problem, clearly demonstrated by our results. Previous attempts to produce *Haematococcus pluvialis* exclusively in open ponds met repeatedly with failure (Bubrick 1991), but the coupled system provided continuous, uninterrupted production (Figure 2).

The reasons that explain successful production of *H. pluvialis* in a coupled cultivation system apply to other species that cannot be produced in open ponds alone. The key to success is to reduce the residence time in open ponds, where cultures are susceptible to contamination. This can be done only by providing a continuous supply of uncontaminated inoculum in large volume, which requires industrial scale photobioreactors. Our results demonstrate that, even when photobioreactor cultures are maintained under light-limited conditions that favor relatively low growth rates (Table V), they occupy a minor fraction of the area required for the entire cultivation system. The most rapid growth rates occur in the open ponds, allowing for a very short residence time and thus avoiding contamination in what represents the majority of the cultivation system on an areal basis.

4.2. MAXIMUM OIL PRODUCTION IN THE COUPLED CULTIVATION SYSTEM

Many species of photosynthetic microbes have higher oil content and intrinsically higher growth rates than *Haematococcus pluvialis*, and thus would be better candidates for industrial oil production. Higher temperatures generally favor species with the highest growth rates (Eppley 1972) but, at a given temperature, most species share the same environmental requirements. They require abundant light, ample nutrients, and a pH that is characteristic of the medium: approximately 7.0 for freshwater or 8.0 for seawater. Thus, many species will eventually proliferate

simultaneously in a pond that is open to the atmosphere. Industrial production of a single species, selected for its oil production rate, demands a cultivation system that is closed to the atmosphere. The coupled system minimizes cost. In a coupled system, photobioreactors provide a continuous source of single-species culture in ample quantity to inoculate the open ponds, allowing the batch cultures in open ponds to exhaust the nutrient supply in a short time, thus avoiding the perils of contamination by other species. Just as this approach is effective for *H. pluvialis*, so it should be effective for species with higher oil content and higher growth rates. Here we estimate the rates of oil production that could be achieved by such other species.

Much of the early research on oil production from photosynthetic microbes focused on the search for species with high oil content. In general, those species with the highest oil content (e.g. *Botryococcus braunii*) grow most slowly (Wolf 1983; Wolf et al. 1985), resulting in low rates of oil production. Nutrient deprivation may result in decreased oil content (e.g. *Dunaliella salina*, Tornabene et al. 1980), but more often leads to increased oil accumulation which, accompanied by nutrient-limited growth rates, results in lower oil productivity (Thomas et al. 1984a,b). Similarly, light limitation can lead to a substantial increase in oil content, but also limits growth so that the result is lower oil productivity overall (Fábregas et al. 2004).

Maximum rates of oil production will result from a combination of high oil content and high rates of biomass production. In general, the highest rates of oil productivity occur under nutrient-sufficient conditions, under which some species have an oil content of up to 38% (Table VI). In the batch culture “grow-out” approach we suggest, nutrient depletion occurs very rapidly, favoring oil accumulation that results in higher rates of oil productivity. The values in Table VI thus represent lower bounds of the oil content that might be expected in rapidly growing cultures.

Maximum photosynthetic efficiency, or the ratio of conversion of photosynthetically active radiation (PAR) to biomass free energy, was estimated from laboratory measurements on *Chlorella* by Pirt et al. (1980) to be as high as 47%. This estimate, which would result in yields of 120–130 g m⁻² d⁻¹, was generally met with “utter disbelief” at the time (Richmond 2000). More typical estimates have placed the maximum potential photosynthetic efficiency in the range of 10 to 20% (Radmer and Kok 1977; Kirk 1994). Such values have already been attained in large-scale cultures exposed to natural sunlight: 20% for *Chlorella* (Tamiya 1957), 15% to 20% for *Phaeodactylum tricornutum* (Acién Fernández et al. 1998; Hall et al. 2003), and 19% for *Tetraselmis suecica* (Laws et al. 1986). We would agree with Richmond (2000) and Pirt et al. (1980) that substantially higher values are possible.

The rate of oil production in a coupled cultivation system could be substantially greater than we achieved with *Haematococcus pluvialis*. Higher rates are achievable now by simply using species with proven greater oil content and higher photosynthetic efficiency. We demonstrate the potential for achievable oil production (Table VII) based on three justifiable assumptions: (1) an increase in

TABLE VI

Oil content, as a percentage of dry weight, for a representative variety of marine and freshwater microalgae grown under nutrient-sufficient conditions

Species	Lipid (%)	Reference
<i>Chlorella emersonii</i>	29	Illman et al. (2000)
<i>Chlorella minutissima</i>	31	Illman et al. (2000)
<i>Chlorella sorokiniana</i>	20	Illman et al. (2000)
<i>Chlorella vulgaris</i>	18	Illman et al. (2000)
<i>Dunaliella salina</i>	14.4	Zhu and Lee (1997)
<i>Dunaliella primolecta</i>	23.1	Thomas et al. (1984b)
<i>Isochrysis galbana</i>	21.9–38.5	Fidalgo et al. (1998)
	26.8	Zhu and Lee (1997)
	28.5	Thomas et al. (1984a)
<i>Nannochloropsis</i> sp.	33.3–37.8	Fábregas et al. (2004)
	30	Zhu and Lee (1997)
<i>Nitzschia closterium</i>	27.7	Zhu and Lee (1997)
<i>Phaeodactylum tricornutum</i>	19.8	Thomas et al. (1984a)
<i>Tetraselmis suecica</i>	20–30	Otero and Fábregas (1997)
	23.1	Thomas et al. (1984b)

photobioreactor biomass productivity, (2) photosynthetic efficiency of 20%, and (3) oil content of 35%. Here we examine each of these assumptions in detail.

- (1) *Increase photobioreactor productivity from 10.2 gm⁻² d⁻¹ to 18.6 gm⁻² d⁻¹.* This assumption suggests a photosynthetic efficiency of slightly more than 5%, which is well within the bounds of observed values (e.g. Morita et al. 2002; Hall et al. 2003). Such an improvement would reduce the relative area occupied by photobioreactors, thus increasing average areal productivity of the entire production system. For the same size photobioreactor, pond area almost doubles, from 417 m² to 760 m², allowing initial pond concentrations to remain identical to those we used for *Haematococcus pluvialis* (i.e. 38 g m⁻³, Tables V and VII). Even with the increase in productivity, photobioreactor cultures will still be light-limited. Inoculum from the photobioreactor will still be exposed to high light intensity in open pond batch culture, and this may further promote oil production. There is evidence, at least for *Haematococcus pluvialis*, that initial exposure to high light intensity results in a significant enhancement of the rate of oil synthesis without impacting growth rate (Zhekisheva et al. 2002).
- (2) *Photosynthetic efficiency of 20%.* Photosynthetic efficiencies of 20%, equivalent to approximately 70 g m⁻² d⁻¹, have already been achieved in open pond or raceway cultures of *Chlorella* (Tamiya 1957), *Phaeodactylum tricornutum* (Ación Fernández et al. 1998) and *Tetraselmis suecica* (Laws et al. 1986). The

TABLE VII

Projected yields of oil from photosynthetic microbes in a coupled system of photobioreactors and open-pond batch cultures assuming (1) an increase in photobioreactor biomass productivity to $18.6 \text{ g m}^{-2} \text{ d}^{-1}$ compared to the reference case of $10.2 \text{ g m}^{-2} \text{ d}^{-1}$ (Table V); and, in the open-pond batch cultures, (2) a photosynthetic efficiency of $\sim 20\%$, and (3) oil content of 35%

Cultivation system & data set	Initial		Growth rate (d^{-1})	Growth period (d)	Final		Oil		Biomass production ($\text{g m}^{-2} \text{ d}^{-1}$)	
	concentration (g m^{-3})	concentration (g m^{-2})			Initial biomass (kg)	concentration (g m^{-3})	concentration (g m^{-2})	Harvest biomass (kg)		production (%)
Photobioreactor	396	53.2	9.90	0.35	n/a ^a				3.47 ^b	18.6
Pond	38	4.6	3.47	2.80	1	625	75.0	35.0	24.6	70.4
Current maximum pond	38	4.6	1.90	1.87	1.3	432	51.8	25.0	9.09	36.4

The reference pond size is increased from 417 to 760 m^2 to allow initial concentrations to remain identical to those currently used. Data on the current maximum pond performance with *Haematococcus pluvialis* are reproduced from Table V for reference.

^aPhotobioreactors operate continuously.

^bBiomass harvested from the photobioreactor is used to inoculate ponds.

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main reason why such high photosynthetic efficiencies have not been sustainable is due primarily to the inherent failure of open-air cultivation systems to maintain uncontaminated cultures for long periods. The strategy we propose avoids this possibility because pond batch cultures would be maintained only for a single day. Contamination by unwanted species under these circumstances is virtually impossible because (1) if the culture inoculum from the photobioreactor is pure, no contaminant will have the potential to compete on a 1-d time scale, and (2) ponds can be sterilized at night, between batches.

- (3) *Oil content of 35%*. We have chosen a target oil content of 35% even though higher values have been attained (Table VI). Oil content in this range has already been observed in nutrient-sufficient cultures of some species, in particular for *Nannochloropsis* sp. (Fábregas et al. 2004) and *Isochrysis galbana* (Fidalgo et al. 1998).

The model pond (Table VII) is initialized with the same starting biomass concentration, and thus the same light conditions, as *Haematococcus pluvialis*. However, as a result of greater photosynthetic efficiency and slightly greater oil content, the projected yield of $70.4 \text{ g m}^{-2} \text{ d}^{-1}$ at 35% oil content results in an oil production rate from the pond system of $24.7 \text{ g m}^{-2} \text{ d}^{-1}$, or $90.1 \text{ toe ha}^{-1} \text{ yr}^{-1}$. Allowing for the additional contribution of photobioreactors that now occupy 19.7% of the total area, this scales to $72.4 \text{ toe ha}^{-1} \text{ yr}^{-1}$ for the entire production system. Assuming a specific gravity for biodiesel of 0.87 yields an energy density of 44.2 GJ/toe and, assuming 100% extraction efficiency, the corresponding yield is projected at $3201 \text{ GJ ha}^{-1} \text{ yr}^{-1}$. We emphasize that this energy yield is for extracted oil only. Dry biomass yield of $70.4 \text{ g m}^{-2} \text{ d}^{-1}$, prorated for a photobioreactor area of 19.7%, corresponds to an annual yield of 206 tonnes $\text{ha}^{-1} \text{ yr}^{-1}$ which, at an energy content of 20 GJ/tonne, is equivalent to $4127 \text{ GJ ha}^{-1} \text{ yr}^{-1}$. The projected yield per unit area of biofuels from photosynthetic microbes is therefore one order of magnitude greater than any potential source based on terrestrial plants (Table III). In fact, even the residual waste product that would remain after oil extraction, $926 \text{ GJ ha}^{-1} \text{ yr}^{-1}$, is more than twice as productive as any terrestrial plant source (Table III). If we project such yields to a global scale, as has been done for terrestrial plants, then the oil-based microbial biofuel needed to satisfy projected demand of 300 EJ yr^{-1} would require only 95 Mha, or 7.3% of the Earth's estimated surplus arable land in 2050.

4.3. FURTHER IMPROVEMENTS IN PRODUCTIVITY

Even greater oil production rates may be achievable than those we have projected based on currently proven oil content and photosynthetic efficiencies. We cannot estimate precisely the results of such further improvements, but the direction of current research indicates type of improvements that might be made. Major advances will result primarily from biological research, in some cases complemented by engineering, especially in the area of fluid mechanics.

4.3.1. *Pure Engineering Solutions*

It is now generally recognized that maximum photosynthetic efficiency is a function of the average photon irradiance per cell, I_{av} , a factor that is influenced not only by incident light, but also by the concentration of cells, the optical path length or depth of the culture, and by the frequency of alternating exposure to high and low light conditions within the culture (Janssen et al. 2003; Richmond et al. 2003). Turbulence in the culture medium also plays an important role because it determines the frequency of exposure to alternating light conditions. In essence, the practical challenge is to expose cells to the highest possible light intensity without causing photoinhibition, which in turn depends upon the state of photoacclimation (Falkowski and LaRoche 1991).

Considerable effort has gone into the design of photobioreactors that would maximize photosynthetic efficiency (e.g. Hu et al. 1996a; Morita et al. 2002; Janssen et al. 2003). However, for the production of biofuels, photosynthetic efficiency is best optimized in open pond batch cultures employed as a second stage in the production process. First, the highest photosynthetic efficiencies recorded to date have been achieved in open ponds and raceways exposed to full sunlight of up to $\sim 2000 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ (Tamiya 1957; Laws et al. 1986; Ación Fernández et al. 1998). There is considerable scope for design or process improvements that would further enhance this result. For example, exposure to photon flux density (PFD) of $8000 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ yields an improvement of 50% in productivity compared to that in full sunlight (Hu et al. 1996b); to take advantage of this result requires an engineering solution. One example of a process improvement would be the removal of auto-inhibitory growth factors by medium replacement, which can lead to a four-fold enhancement in areal productivity (Richmond et al. 2003). This particular method could be accomplished by the two-stage cultivation process we developed, in which culture medium from the photobioreactor is significantly diluted upon transfer to the second-stage open-pond batch culture.

There are many ways in which bioengineering could result in significant enhancement of biomass or oil productivity. The following examples serve to illustrate how much has been accomplished to date despite a relatively meager allocation of resources, and suggest that remarkable improvements could be achieved if bioengineering studies were more vigorously pursued.

4.3.2. *Reduction of the Light-Harvesting Pigment Complex*

Reduction in size of the light-harvesting pigment (LHP) complex can lead to greater photosynthetic efficiency at high photon flux density. The photosynthesis of cells with a large complement of LHP does not increase in proportion to light intensity at high PFD because excess excitation energy is dissipated to non-photochemical quenching and heat and, consequently, photosynthetic efficiency is decreased (Lien and San Pietro 1976; Benemann 1989). Model simulations confirm the concept (Sukenic et al. 1987; Melis et al. 1999). Furthermore, recent experiments with mutant strains of two species of microalgae – a

Chlamydomonas perigranulata mutant with a small light-harvesting pigment complex and a phycocyanin-deficient *Synechocystis* mutant – have demonstrated photosynthetic rates that are three- and five-fold higher than wild-type strains, respectively, at the PFD of 2000 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ characteristic of full sunlight (Nakajima and Itayama 2003).

4.3.3. *Enhancement of Oil Biosynthesis*

There has been much research on oil synthetic pathways in higher plants, and those pathways have simply been assumed to be similar in photosynthetic microbes. However, the quantity and quality of oils undergo major changes during nutrient deprivation in these organisms; this does not happen in higher plants. Relatively little has been done to enhance, let alone to understand, oil biosynthetic pathways and regulation in photosynthetic microbes. One of the first attempts to use a genetic transformation system directed at enhancing oil production succeeded in making the transformation (Roessler 1988a,b), but did not result in a detectable increase in oil production (Dunahay et al. 1995, 1996). However, there have since been many more successful species transformations made in photosynthetic microbes (León-Bañares et al. 2004), suggesting that new methods and strategies might be successfully applied in a renewed effort.

4.3.4. *Engineering Rubisco for Enhanced Photosynthetic Efficiency*

It has long been recognized that the enzyme ribulose 1,5-disphosphate carboxylase/oxygenase (Rubisco), which catalyzes the first step in photosynthetic CO_2 fixation and respiratory carbon oxidation, imposes the primary rate-limiting step on plant productivity (Salvucci et al. 1985). Rubisco's notorious inefficiency has led to the suggestion that the enzyme might be improved as a means to increase photosynthetic efficiency (Spreitzer and Salvucci 2002). Much of the research effort has been targeted at higher plants with the goal of improving agricultural yields, but one of the more promising recent developments has been accomplished with photosynthetic microbes. Using the photosynthetic bacterium *Rhodobacter capsulatus* as host, Smith and Tabita (2003) successfully introduced Rubisco genes from the cyanobacterium *Synechococcus*, obtaining a positive mutant that exhibited a significant improvement in growth rate. The initial interpretation of these results, that the mutant enzyme had an improved affinity for CO_2 , was not borne out by further examination. Nonetheless, this study demonstrates the potential for using photosynthetic microbes in a system of random mutagenesis and biological selection that yields changes in the properties of Rubisco. The benefits of a more efficient Rubisco are obvious, and demand a more concerted research effort targeted at photosynthetic microbes in particular.

In summary, oil production rates of $>3000 \text{ GJ ha}^{-1} \text{ yr}^{-1}$ from photosynthetic microbes should be achievable now using species with demonstrated performance characteristics in a coupled cultivation system. If the experience with higher plants in agricultural biotechnology may serve as an indicator of the potential

for enhancing yields, then further improvements appear equally likely for photosynthetic microbes.

4.4. ECONOMICS OF BIOFUEL PRODUCTION FROM PHOTOSYNTHETIC MICROBES

Several estimates of the cost of oil production from photosynthetic microbes place the cost in the range of \$39 to \$127 per barrel, with variations attributable primarily to assumptions of productivity (Table IV and Benemann et al. 1982; Benemann and Oswald 1996). These models were based on a production system consisting entirely of open ponds, and using stack gas from fuel-burning power plants as the source of CO₂. The most recent of these estimates arrived at a cost of \$67/bbl and \$39/bbl at productivities of 30 and 60 g (dry weight) m⁻² d⁻¹, respectively. Both models assumed 40% oil content, yielding oil production rates of 43.8 and 87.6 toe ha⁻¹ yr⁻¹ (Table IV). By comparison, we arrive at a projected production rate of 72.4 toe ha⁻¹ yr⁻¹ for a coupled cultivation system, taking into account the area occupied by photobioreactors. Assuming no difference in the cost of photobioreactors and open ponds, our production estimate would yield a cost of \$49/bbl which, adjusted for inflation, yields a cost approximately \$56/bbl (2003 US\$). At this writing (October 2004), crude oil is trading at more than \$55/bbl.

There is, of course, a difference in capital cost between open ponds and photobioreactors. Hallenbeck and Benemann (2002) estimated photobioreactor capital costs at approximately US\$100 m⁻², which agrees well with our own experience from having built several dozen photobioreactors at various scales up to 25,000 l. We emphasize that photobioreactor technology remains very much in the prototype stage, and that significant cost improvements are bound to occur as the technology is further developed. Nonetheless, we can use the value of US\$100 m⁻² to estimate the cost of renewable oil production in a coupled cultivation system.

In the coupled cultivation system for which we estimated a production rate of 72.4 toe ha⁻¹ yr⁻¹ (Section 4.2), 80.3% of the production facility area consists of open ponds, and 19.7% of photobioreactors. Benemann and Oswald's (1996) estimate for the \$39/bbl production facility was based on total annual costs of \$29,370 ha⁻¹, comprised of net operating costs at \$15,270 ha⁻¹ and a capital charge, at a 15% depreciation rate, of \$14,100 ha⁻¹. Interpolating from the depreciation rate, total capital cost for the open pond system is \$94,000 ha⁻¹, or \$9.40 m⁻² – about one tenth the capital cost of current photobioreactor technology. If we now prorate capital costs for the fractional areas occupied by open ponds and photobioreactors, the average hectare will incur a cost of \$74,482 for open ponds and \$197,000 for photobioreactors, for a total capital cost of \$272,482 ha⁻¹. Assuming straight-line depreciation at a rate of 15% per annum, this yields an annual capital charge of \$40,872 ha⁻¹. Assuming that net operating costs remain constant at \$15,270 ha⁻¹, the total annual costs would be \$56,142 ha⁻¹, equivalent to \$74/bbl. Adjusting for inflation since 1996 yields cost of about \$84/bbl.

A 75% reduction in photobioreactor cost would have a substantial impact, yielding a current cost of \$51/bbl. Based on our own experience, this could be achieved by quadrupling the capacity of the modular photobioreactor to 100,000 l, a not unreasonable proposition. The overall cost reduction arises from the very low proportional cost of tubing that comprises the photobioreactor cultivation chamber, which costs only \$1 m⁻². We believe that other significant cost reductions will also occur as the technology is further developed.

4.5. CONCLUSIONS

The main strategy now being considered for biological mitigation of anthropogenic CO₂ relies entirely on terrestrial plants, whether for carbon storage or biofuels production (IPCC 2001b; Berndes et al. 2003). The second biological mitigation strategy still being given some attention is the possibility of stimulating oceanic primary production by the addition of iron (Martin 1990, 1991), but attendant uncertainties and risks of such a global experiment suggest that policy makers will be reluctant to adopt this strategy (Chisholm et al. 2001). A third strategy, relying on biofuel production by oil-rich photosynthetic microbes, was studied intensively by major government-sponsored research programs in Japan and the USA and then largely abandoned in the late 1990s, after investments of US\$117 million and US\$25 million, respectively. Neither program was able to design or operate sustainable production technology beyond the laboratory scale.

Previous research and development on production systems for photosynthetic microbes focused either exclusively on photobioreactors or exclusively on open ponds (Lee 2001). Photobioreactors provide controlled conditions that yield reproducible product at high rates, but they are expensive. Open ponds are far less costly, but are so easily contaminated that after more than 50 years of repeated attempts, no more than three species proved amenable to large-scale cultivation.

The coupled cultivation system we developed takes advantage of the benefits of both photobioreactors and open ponds, while avoiding their disadvantages. Our success with commercial scale production of *Haematococcus pluvialis* demonstrates that photobioreactors are essential to the sustainable production of photosynthetic microbes that cannot be cultivated reliably in open ponds. The photobioreactor system provides for a continuous supply of high-quality culture inoculum to the open ponds. Rapid growth rates in the ponds allow an extremely brief residence time, thus avoiding the potential for contamination. We operated the coupled cultivation system continuously for more than one year at 2-ha scale and, for *H. pluvialis*, achieved an average biomass energy production of 763 GJ ha⁻¹ yr⁻¹, with an oil production rate of 422 GJ ha⁻¹ yr⁻¹. The maximum value of biomass energy production we achieved would have yielded an annual rate of 1836 GJ ha⁻¹ yr⁻¹. These values are substantially greater than any rates of bioenergy production projected for terrestrial plants, most of which fall in the range from 50 to 400 GJ ha⁻¹ yr⁻¹ (Table III).

We show that bioenergy production rates of $>4100 \text{ GJ ha}^{-1} \text{ yr}^{-1}$ could be achieved now in the coupled cultivation system with microbes that have greater proven oil content and photosynthetic efficiency than *H. pluvialis*. The oil production rates would be in excess of $70 \text{ toe ha}^{-1} \text{ yr}^{-1}$, corresponding to energy production of $>3200 \text{ GJ ha}^{-1} \text{ yr}^{-1}$. We estimate the cost of oil production from photosynthetic microbes to be \$84/bbl, assuming that no improvements are made to current production technology, and that no value is attributed to the excess bioenergy production of more than $900 \text{ GJ ha}^{-1} \text{ yr}^{-1}$ contained in the non-oil fraction of the biomass produced. It is unreasonable to suppose that no improvements will be made to the technology. We provide one example, a quadrupling of individual photobioreactor capacity, that alone could reduce production costs to almost \$50/bbl. Enhancements of photosynthetic efficiency or oil biosynthetic pathways via strain selection or bioengineering have the potential to further reduce costs.

We suggest that the proof of concept demonstrated in this paper merits further attention from scientists and policy makers alike. The cost of oil production from photosynthetic microbes might now be only marginally competitive with fossil sources of petroleum. Furthermore, the microbial production process may be optimal only in tropical or subtropical environments. However, over the longer term renewable oil will become increasingly attractive as the costs of exploration and extraction of fossil fuels continue to increase. The recent decision of Russia to agree to the Kyoto Protocol and, in so doing, provide for ratification of the international treaty, creates additional incentive to reduce stack gas emissions of CO₂, a process upon which the production of biofuels from photosynthetic microbes explicitly depends. Reliance on biofuels from terrestrial plants to replace current fossil fuel usage equivalent to 300 EJ yr^{-1} would require at least 80% of the Earth's surplus arable land projected to be available by 2050. By contrast, at currently achievable rates of production, photosynthetic microbes would require only 7.3% of that surplus arable land.

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