August 1, 2003

Minutes of the 3rd Technical Meeting of the International Network on Biofixation of CO_2 and Greenhouse Gas Abatement with Microalgae.

April 28-29th April 2003, Berkeley, California.

INTRODUCTION. The major objectives of this Meeting were to review progress and promote cooperation and collaborations among the Network Member Organizations and their R&D projects and to discuss plans for the second year of the Network. The R&D projects presently carried out or supported by the Member Organizations and several proposed projects were presented and discussed. The next Network meeting will be September 29, 2003, in Paris, France, at the Concorde Lafayette Hotel, just preceding the meeting of the Executive Committee of the IEA Greenhouse Gas R&D Programme to be held at the same venue.

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A. WELCOMES AND INTRODUCTIONS

1**. Dr. Bailey Green, LBL, Welcome to LBL.**
Dr. Green welcomed the participants to this meeting to the Lawrence Berkeley Laboratory, a U.S. Dept. of Energy National Laboratory managed by the University of California. LBL is currently carrying out a number of greenhouse gas abatement projects, in particular carbon sequestration projects, under the direction of Dr. Sally Benson. Dr. Green works with Prof. W.J. Oswald on microalgae wastewater treatment and other applications (see presentation).

2. Dr. Paola Pedroni, EniTecnologie, Chair, Welcome to the 3rd Technical Meeting.
Dr. Pedroni welcomed the participants and reviewed the Network history and activities. The Network was initiated in 2000 with a proposal by the U.S. Dept. of Energy and EniTecnologie to the Executive Committee of the IEA Greenhouse Gas R&D Programme, leading to a Workshop in Monterotondo, Italy, in January 2001 and an organizational meeting in Reston, Virginia, USA, January 2002. The Network started operating in June 2002, with two technical meetings held, in Almeria, Spain, in May, 2002, and Kyoto, Japan, October, 2002. (Minutes available from the Network Manager, John Benemann.) The Network is open to organizations with interest in R&D of microalgal technologies for GHG abatement and currently has eight members*. It is managed through its Steering Committee representing all Member Organizations. The Network is not a R&D funding mechanism, with each member organization carrying out its own R&D agenda. Six Technical Advisers have participated in the Network for the past year in helping to formulate and review R&D plans and objectives**. The goal of the Network is to demonstrate the technical feasibility of GHG abatement through biofixation of CO_2 by microalgae within five years and to initiate practical applications within 10 years. A "Technology Roadmap" was prepared with DOE support by the Network Manager with inputs from the members and technical advisers and completed in January, 2003. The objectives of this 3rd Technical Meeting are to discuss the R&D activities being carried out by the members and plan for future activities.

3. Dr. John Davison, IEA Greenhouse Gas R&D Programme. Management and Budgets.
The Greenhouse Gas R&D Programme, part of the International Energy Agency (IEA), has over a score of member countries and participating energy companies, and has carried out technology assessments and engineering-economic analyses of greenhouse gas abatement technologies for the past ten years. The microalgae Network is one of several interest groups being managed by the Programme (see www.ieagreen.org.uk). Dr. Davison discussed the finances of the Network: based on the contributions of $9,000 from each of the eight members, the budget for the first year was $72,000 and, as approved by the members, was allocated as follows: $28,000 for support of the Network Manager, Dr. Benemann ($4,000/mo, for seven months); $15,000 for the Technical Advisers (a honorarium of $1,000 per technical meeting attended), $21,000 for the three technical meetings, $6,000 for the IEA Greenhouse Gas R&D Programme, and $2,000 for contingencies. With the current meeting the budget has been essentially fully expended.

*The current Network members are: Arizona Public Services, ENEL Produzione Ricerca, EniTecnologie, EPRI, ExxonMobil (membership not continued after 6/1/03), Gas Technology Institute, Rio Tinto, and U.S. Department of Energy-National Energy Technology Laboratory.
** The Technical Advisers are: Dr. Amha Belay, Earthrise Farms (USA); Prof. David Brune, Clemson Univ. (USA); Dr. Gerald Cysewski, Cyanotech (USA); Mr. Yoshi Ikuta, Consultant (Japan); Prof. Mario Tredici, Univ. Florence (Italy); Dr. Joseph Weissman, SeaAg, Inc. (USA).

4. Dr. John R. Benemann, Network Manager. Agenda and Technology Roadmap.
Dr. Benemann thanked the attendees for their participation and, in particular, Dr. Green for hosting the meeting at LBL and having led the tour to the St. Helena wastewater treatment plant in Napa Valley on the previous day, Sunday. He briefly introduced the Technology Roadmap developed for the Network with support from the U.S. Dept. of Energy, National Energy Technology Laboratory, and with inputs from the Steering Committee Members and Technical Advisers, as well as others. The fundamental issue in greenhouse gas abatement with microalgae is that current technology does not allow sufficiently low-cost production of algal biomass for conversion to fossil fuel substitutes, even assuming low-cost, large-scale, open pond systems and very high productivities, neither of which has yet been demonstrated in practice. Thus, at present microalgae technologies require the co-production of renewable fuels with higher-value (than fossil fuels) but large-volume co-products or with other services, such as water purification. The Technology Roadmap identified wastewater treatment and nutrient recovery from polluted waters, along with co-production of animal feed, organic fertilizer and specialty chemicals (e.g. lubricants, polymers, etc.) as of high enough value and large enough markets to make significant impacts in greenhouse gas abatement. In all cases, only large, open, unlined ponds can be considered for such applications for economic reasons. The key technology issues in microalgae culture are how to maintain desired species in large open ponds, how to maximize productivity, how to harvest the algal cells, and how to convert the biomass to fuels and other useful products. Although engineering issues of large-scale ponds, such as hydraulics (such as sediment suspension), as well as power plant flue CO_2 transfer, are also of interest, they are less critical than the biological R&D issues. The objective of this 3rd Technical Meeting was to review current and planned R&D projects by Members and others potential participants and help to foster integration, coordination and collaborations among these projects and researchers.

B. INTRODUCTIONS OF ATTENDEES.

Dr. David Barr, Rio Tinto Technical Services (Member). Rio Tinto is a large international mining company with world-wide operations, with a small staff dealing with biotechnological issues. The company is a major coal producer in the U.S. (through Kennecot Mining) and is interested in CO_2 abatement issues. It is sponsoring a project with Maxygene /Codexis (Redwood City, Calif.) on the improvement of the CO_2-fixing enzyme, RuBisCo in microalgae (see presentation) as well as CO_2 mineralization by algal cultures. Thus the interest by Rio Tinto in the Network, as a potential avenue to explore and develop practical applications of this field.

Dr. Amha Belay, Earthrise Farms (Technical Adviser). Earthrise Farms is the largest microalgae farm in the U.S., possibly the world, in terms of output. It is located at the southern end of the Salton Sea and produces *Spirulina*, used as a human food supplement and animal feed. Most of its *Spirulina* production ponds (some twenty overall) are somewhat below one acre in size and are plastic lined. Two larger ponds are unlined, several acres in size and use single paddle wheels. The production of *Spirulina* has considerable scope for improvements.

Dr. Dave Brune, Clemson University, (Technical Adviser). Clemson University has pioneered the use of microalgae in fish aquaculture, developing a patented "Partitioned Aquaculture System" (PAS) that uses high rate ponds for waste treatment and oxygen generation for fish ponds, in addition to allowing co-culture of herbivorous species. This process is also being applied at the Salton Sea project, being carried out by Kent SeaTech Corp. (see presentation).

Dr. Gerald Cysewski, Cyanotech Corp. (Technical Advisor). Cyanotech produces *Spirulina* and *Haematococcus* pluvialis, a source of natural astaxanthin in over seventy ponds in Kona, Hawaii. One of the aspects of interest in the context of greenhouse gas abatements, is that the Cyanotech facility operates a small power plant that uses biodiesel for the generation of electricity, with the flue gas captured and recycled used for the algal production (see Monterotondo Workshop Report and Minutes of 2nd Technical Meeting in Kyoto for details). Cyanotech Corp. is also interested in the development of organic fertilizer using nitrogen-fixing cyanobacteria and other aspects of relevance to the Network research.

Pradeep Dadhich, TERI India (Observer). TERI, formerly Tata Energy Reserarch Institute, is a research organization with several hundred scientists developing and applying appropriate and renewable energy technologies in India, with a strong interest in biomass energy systems, including anaerobic digestion. TERI represents India on the Executive Committee of the IEA Greenhouse Gas R&D Programme. India has considerable expertise in microalgae culture, including R&D in biofertilizer production using N_2-fixing cyanobacteria for rice paddy inoculation, with a culture collection established. Several commercial microalgae production facilities are also operating in India, in particular the Muragappa Group in Tamil Nadu (Southern India) that has a production plant for *Spirulina* and beta-carotene from *Dunaliella salina*. In relation to the Network, TERI is considering initiating R&D activities related to wastewater treatment and biofuel recovery with algae ponds, in particular as these could be applied to wastewater treatment.

Patrick Hallenbeck, Universite de Montreal (Invited Researcher). Dr. Hallenbeck was invited to present a brief discussion of microalgae hydrogen production, a major focus of R&D interest in the U.S. and elsewhere, and which Prof. Hallenbeck studied in the past. He is currently studying fermentative bacterial metabolism at a biochemical and genetic level (see presentation).

Qiang Hu, University of Arizona (Invited Researcher). Dr. Hu main focus of research has been in the use of photobioreactors and physiology of high density microalgae cultures and applications to environmental remediation. He is now working with Prof. Milton Sommerfeld and Universal Entech, LLC (Dan Musgrove, see below) on an Arizona Public Services funded project on greenhouse gas abatement with microalgae (see presentation).

Michael Huesemann, Pacific Northwest National Laboratory (Invited Researcher). Dr. Huesemann started a research project funded by the U.S. Department of Energy National Energy Technology Laboratory a little over a year ago on the subject of investigating the maximum growth rate-productivity relationship between different algal species and strains. This is a basic R&D project that is of relevance in the development of both inoculation systems, which are based on fast growth rates, and biomass production, where light limited growth is to achieve the highest solar conversion efficiency (see presentation).

Evan Hughes, EPRI. (Member**)**. EPRI (until recently The Electric Power Research Institute) is the research organization for the U.S. and now the global power industry. EPRI has had a long history of R&D in bioenergy, going back 25 years, including several projects related to microalgae greenhouse gas abatement. EPRI has continuing interest in this area and is supporting the Network to evaluate future participation in proposed projects.

Yoshi Ikuta, Consultant (Technical Advisor). Until retirement, Mr. Ikuta worked for Mitsbushi Heavy Industries, where he managed the R&D efforts of the several Japanese electric utilities efforts in microalgae greenhouse gas abatement, mostly using small open ponds. This was a separate and smaller program from the much larger ten year effort (over $100 million program in direct costs alone) Japanese government (METI)-funded R&D program, which ceased by 2000 due to the uneconomic nature of these photobioreactor-based processes. Mr. Ikuta has also been associated with SeaAg, Inc. and helped establish a clam operation in Japan. Over the past year he has worked on making the Network known in Japan and rekindling interest in this technology, but that has proven difficult. One utility in Japan, Chuoku Electric, is currently initiating a project on microalgae for greenhouse gas abatement.

Michael Massingill, Kent SeaTech Corp. (Invited Researcher). Kent SeaTech is an aquaculture production company in California that commercially produces striped bass near the north shore of the Salton Sea. The company has worked with Prof. Brune on several projects, including his Partitioned Aquaculture System, for which two pilot-scale (3,000 m^2) high rate ponds were installed at their facility. These ponds are now used for a Salton Sea Authority funded project, with Dr. Massingill as Principal Investigator, that aims at removing nutrients from agricultural drainage waters before these flow into the Salton Sea, (See presentation by Dr. Brune).

Blaine Metting, Pacific Northwest National Laboratory (Observer). PNNL is one of the U.S. DOE laboratories that is carrying out the carbon sequestration program of the Office of Science, with Dr. Metting managing this effort at PNNL. Dr. Metting has also been involved in the past in microalgae research and commercial applications for over twenty-five years, in particular the production and applications of soil microalgae that can enhance soil fertility. Dr. Metting is interested in the Network because of its relevance to greenhouse gas abatement and the ongoing R&D by PNNL in this area (see Michael Huesemann presentation).

Dan Musgrove, Universal Entech LLC (Observer). This Company is a small business involved in the development and application of environmental technologies, including, for example, anaerobic digestion of animal wastes. EnTech is working together with Arizona State University and Arizona Public Services on the application of photobioreactors for microalgae waste nutrient recovery and co-production of animal feeds (see presentation by Dr. Qiang Hu).

Duane Pilcher, Arizona Public Services (Member). Arizona Public Services (APS) is a large U.S. electricity utility that carries out considerable in-house R,D&D of renewable energy technologies. There has been an interest at APS for several years in the use of microalgae for greenhouse gas abatement, with first contacts established almost a decade ago with NREL (National Renewable Energy Laboratory) and APS is sponsoring R&D at Arizona State University (see presentation by Dr. Quiang Hu). APS has joined the Network to further the development of this technology with the view of potential applications in the APS service area.

Juergen Polle, Brooklyn College (Invited Researcher). Dr. Polle was until recently at the University of California Berkeley where he carried out research on photosynthetic efficiencies by microalgae cultures, specifically through the genetic reduction of the antenna chlorophyll in *Chlamydomonas* (See presentation). He is now at Brooklyn College and is continuing this research. He is participating with Joseph Weissman in a proposal to develop genetically improves strains with high photosynthetic efficiencies and test these in outdoor ponds.

Roger Prince, ExxonMobil (Member). ExxonMobil has an interest in microalgae technologies for their potential for providing alternatives to hydrocarbon products. Specifically ExxonMobil has sponsored R&D of Botryococcus *braunii*, a unique alga that produces interesting long-chain hydrocarbons of potential interest as lubricants. (See presentation). ExxonMobil has recently funded a large R&D program at Stanford University in the area of greenhouse gas abatement and has therefore curtailed its in-house and sponsored R&D activities in this area, resulting in it withdrawing from participation in the Network effective June 1, 2002.

Paul Roessler, Dow Chemical Co. (Invited Researcher, Observer). Dr. Roessler has a background in microalgae R&D through his long association with the Solar Energy Research Institute (now the National Renewable Energy Laboratory, Golden, Colorado, a USDOE national laboratory), where he was involved mainly on developing genetic approaches to oil production by microalgae, as part of the "Aquatic Species Program". He continued related work at Monsanto Corp., for the production of specialty oils from microalgae by heterotrophic fermentations. For the past few years he has been working at Dow Chemical Co., managing R&D programs on the production of vegetable oils. He participated in this meeting to provide his perspectives on the potential of genetic applications to microalgae cultures and track the Network activities as they may provide new sources of vegetable oils.

Milton Sommerfeld, Arizona State University (Invited Researcher). Dr. Sommerfeld was also a participant in the "Aquatic Species Program", mainly through the isolation and characterization of many algal species and strains collected from various locations throughout the U.S. Southwest. Currently he is working with Qiang Hu and Dan Musgrove on the Arizona Public Services funded project (see presentation by Dr. Hu).

Vipul Srivastava, Gas Technology Institute (GTI) (Member). GTI has a history of R&D in the field of biotechnology as it applies to methane gas production (anaerobic digestion) and other renewable fuels, including biomass gasification. GTI is also working on CO2 capture and sequestration technologies for greenhouse gas abatement. GTI's interest is in the possibility offered by microalgae systems to produce renewable fuels, in particular through anaerobic digestion and other fermentations as part of their role in wastewater treatment. GTI is a member of the network with the objective of advancing this technology and increasing the potential renewable energy resource.

Joseph Weissman, SeaAg, Inc. (Technical Adviser). Dr. Weissman was the Principal Investigator in a number of the key projects of the Aquatic Species Program (ASP) of NREL including operation of the Algae Test Facility in Roswell New Mexico. SeaAg, Inc. is a clam aquaculture company based on a diatom cultivation process developed by Dr. Weissman and the company also carries out R&D and consulting in microalgae technologies. Currently Dr. Weissman is working on a "Phase I Small Business Innovative Research" project funded by the U.S.Department of Energy (see presentation). (*Note added after meeting: in June SeaAg, Inc., with Dr. Joseph Weissman as Principal Investigator, were awarded a two-year, $600,000 Phase II SBIR U.S. Department of Energy project to work, with Prof. Juergen Polle, Brooklyn College, working under a subcontract on the isolation and demonstration in outdoor ponds of high efficiency truncated antenna mutants.)*

C. **SUMMARIES OF TECHNICAL PRESENTATIONS OF CURRENT PROJECTS.**

1. Paola Pedroni, EniTecnologie, Milan, Italy. "Microalgae for Greenhouse Gas Abatement – The EniTecnologie R&D Project"

EniTecnologie is the research arm of Eni, the major Italian oil and gas company, with laboratories in Milan and Monterotondo. EniTecnologie is carrying out environmental R&D projects in a number of areas, including biotechnology. For example, recently EniTecnologie completed an R&D project in conjunction with the Japanese RITE Program on H_2 production from organic wastes by photosynthetic bacteria using outdoor photobioreactors. In that project, genetic engineering techniques were also applied to produce mutants that eliminated reactions competing for reductant in H_2 production, with the mutants shown to have improved H_2 evolution in laboratory experiments and with outdoor photobioreactors. That work led to an interest in photobiological processes, specifically microalgae systems for CO_2 fixation and greenhouse gas abatement.

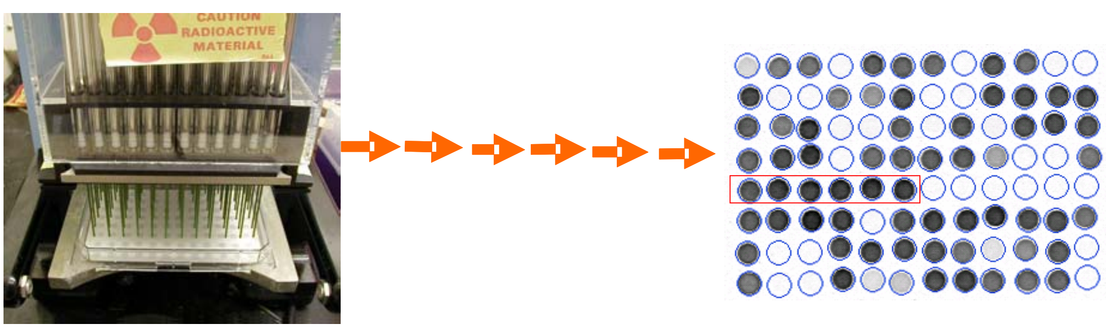
In 2000 EniTecnologie joined with the U.S. Department of Energy in proposing to the Executive Committee of the IEA Greenhouse Gas R&D Programme the "International Network" and hosted the initial Workshop in Monterotondo, near Rome, in January 2001, where the potential of microalgae for greenhouse gas abatement was discussed by a group of over thirty researchers and representatives from various energy companies. The broad consensus developed at the Workshop supported this concept encouraged EniTecnologie management to initiate an in-house R&D program in this field, in addition to continuing to assist with the establishment of the Network. The importance of the Network is that it provides a forum for R&D coordination, collaboration and integration, which makes it possible for organizations to work collaboratively on this complex and multifaceted problem, which will require long-term technology development and involve many site-specific applications.

The specific microalgae CO_2 biofixation technology that EniTecnologie is developing involves the capture of flue gas CO_2 from natural gas-fired power plants and recycling this carbon back to the power plant in the form methane gas produced from the anaerobic digestion of the microalgae biomass. The research team at EniTecnologie in Monterotondo is focusing its initial efforts on a specific problem: the achievable productivity of microalgae, using currently available strains, and specifically a comparison of the productivity of open ponds vs. closed photobioreactors operated outdoors. There is a lack of comparative data on this point, and it is crucial to determine if, indeed, ponds have inherently lower productivities than closed photobioreactors and, if so, what factors would contribute to this effect. One decision made was to use photobioreactor designs that receive a similar sunlight intensity and amount as ponds, that is horizontal rather than vertical systems. The specific design chosen was one developed by Prof. Mario Tredici, University of Florence, and adviser to the EniTecnologie project, consisting of slightly sloping tubular reactors with internal gas exchange, as also used for a project in Hawaii. For the open ponds, a standard paddle wheel mixed design was selected. Two ponds, of about 2.5 m^2 each and two tubular photobioreactors, of about 1 m^2 each, were designed and constructed by Prof. Tredici and his team, who also supplied the required control systems. A natural gas combuster is used to provide the flue gas CO_2 to these units. The experimental system is now operating at the Monterotondo facility of EniTecnologie and initial data is being collected with both marine diatoms and green algae. Results from this research will be reported on at the next Network meeting.

2. David Barr, Rio Tinto, Australia "Improved Microbial CO_2 Fixation through Gene Shuffling"

Rio Tinto is one of the world's leading mining companies with worldwide operations and group companies in over 40 countries, employing some 35,000 people with group sales. It is a major coal producer in the U.S., and as part of its concern about environmental issues and long-term business prospect, Rio Tinto is exploring a number of different options and technologies in areas related to greenhouse gas abatement. Rio Tinto is funding ongoing projects on improving the process for biological CO_2 fixation through the genetic modification of the enzyme *RuBisCo*. Maxygen, Inc., a biotechnology company in Redwood City, California, is carrying out the main part of this research through its subsidiary Codexis, using proprietary MolecularBreeding™ techniques to achieve the directed molecular evolution of a more functional enzyme, with greater resistance to O_2 side-reactions (e.g. photorespiration), higher catalytic rates, greater affinity for CO_2, etc. Such improvements would allow to significantly increase the amount of CO_2 being fixed by plants and microalgae, with RuBisCo from microalgae being the specific target of the Maxygen-Codexis work.

RuBisCo is the rate limiting enzyme in the CO_2 fixation pathway, as evidenced by the high content of this enzyme in photosynthesizing organisms, the low turnover numbers and the competition between O_2 and CO_2 for the active site. The lack of a "rational" design for improving RuBisCo makes the Maxygen-Codexis approach of interest: gene "shuffling" (essentially random recombinations) among RuBisCo genes from different organisms, each with somewhat different properties, with the aim of achieving a new genetic sequence with overall better catalytic properties. Libraries of different RuBisCo genes are expressed in *E. coli* and assayed in vitro (in 96 well plates) for CO_2 incorporation rates:



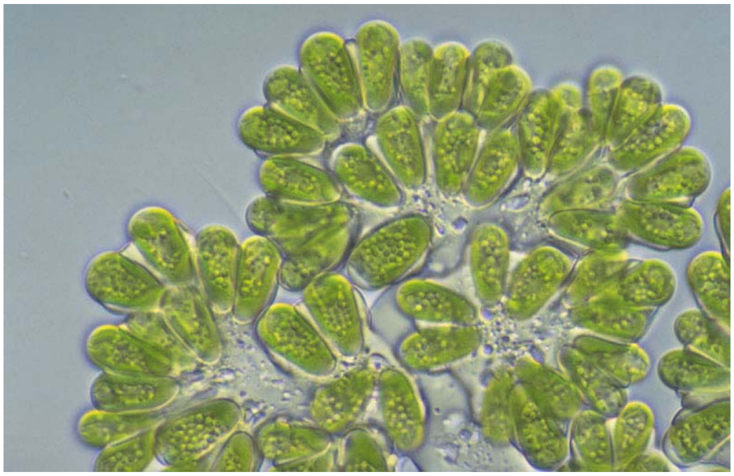
RuBisCo quantization by active site titration: Phosphorimage analysis of $^{14}CO_2$ incorporation (Source: Codexis/Maxygene)

The improved genes are then transferred to *Synechocystis* to replace the wild type enzyme and whole cell CO_2 fixation assayed. This approach has resulted in major improvement in the kcat for RuBisCo, over four -fold above that reported previously for RuBisCo and over five-fold for prior approaches with "rational" protein engineering approaches. Transfer of the improved RuBisCo gene into *Synechocystis* improved the measured whole cell CO_2 fixation rate two-fold with 5% CO_2 in the gas phase. Future work now must establish if such an increased rate of CO_2 fixation can be also achieved under conditions of relevance to algal mass cultures. The application of this technology to increase microalgae CO_2 biofixation for greenhouse gas abatement and for other processes and products is the long-term goal of this project.

3. Roger Prince, ExxonMobil Research and Development Co., Annandale, New Jersey, USA. "*Botryococcus* as an agent for Greenhouse Gas Mitigation?

Dr. Prince reviewed the basic concept of microalgae utilization for greenhouse gas abatement, which could be integrated with natural gas fired power plants to use exhaust gases to grow the algae and then obtain valuable by-products and ferment the remainder of the microalgae biomass to methane gas. Methane fermentations are well established and widely used already in the waste disposal with landfill gas a significant source now, with some industrial applications.

One potential algae of interest in such a scheme is the hydrocarbons producing *Botryococcus braunii*. *B. braunii* is a member of the green algae defined by characteristic cells and colonial growth form and the production of hydrocarbons of various compositions. *B. braunii* is a cosmopolitan organism in oligotrophic (low nutrient) waters.



Botryococcus braunii. Cells typically about 10-15 um and colonies 25-35 um, coalescing into larger assemblages. Photo: www.nies.go.jp/biology/mcc/images/PCD5008/1013-2L.jpg

B. braunii hydrocarbons are of interest in that they are produced in large amounts, often 50% or total dry weight, sometimes as large oil droplets. The chemistry of the hydrocarbons reveal three distinct "races": Race A, producing linear hydrocarbons with C25 to C31 and two or three unsaturated bonds in the middle; Race B, producing unsaturated triterpenes, so-called bottryococcenes, of general molecular weight C30 to H50, and Race C producing a tetraterpene of molecular weight $C_{40}H_{78}$. One reason for interest in *B. braunii* and its hydrocarbons is that it is the source of presently forming "oil deposits" ("coorongite" in Australia) and saturated botryoccocane is found in certain oil deposits. From a biotechnological perspective, the hydrocarbons and possibly some biopolymers made by *B. braunii* could be of interest as fuels and in some cases (e.g. the Race A type hydrocarbons) as higher value (than fuels) lubricants. The mass culture of such algae remains a challenge due to the low growth rates of these algae and there is essentially no work carried out on its outdoor cultivation. This should be a topic for future development under the Network.

4. Dr. Qiang Hu, Arizona State University, Tempe, Arizona, USA.
"Greenhouse Gas Abatement and Environmental Remediation with Microalgae Cultures"

Dr. Hu has carried out research on microalgae photobioreactor designs and physiology of high density cultures, and their applications to production of nutraceuticals and in environmental remediation. For examples, his work has included microalgae-based nitrate removal from high nitrate-contaminated groundwater and cyanobacteria-associated odor and taste problems in drinking waters, two major world-wide problems.

Drs. Qiang Hu and Milton Sommerfeld at ASU are working with support from Arizona Public Services and with Universal Entech LLC, a company specializing in biological processes for waste management, on the applications of microalgae in greenhouse gas abatement. The emphasis of this project is on the use of agriculture runoff and animal wastes for nutrient and energy recovery and biomass production using photobioreactors (200 to 10,000 liters) and anaerobic digestion. Although this project is still in its initial stages, some preliminary results obtained so far are encouraging.

Microalgae represent only a small fraction, ca. 0.2%, of total global plant biomass, but are thought to fix a large portion, as much as half, of the total CO_2 cycled through the biosphere, due to their dominance in the oceans and their very rapid growth (and decay) rates in the environment. Average ocean fixation of C is 1.4 to 2.1 tons of C per hectare per year, while engineered systems can produce 18 to 36 tons of C/ha/year and the potential is for 80-100 t C fixed /ha-yr. The issue is how to achieve the high projected productivities that appear possible from laboratory results and theoretical considerations. The biological and engineering challenges in algal mass culture are fundamentally ones of the supply and utilization of light irradiance in the mass culture system. In open ponds the minimum light path is about 200 mm, vs. 50 mm or less in closed photobioreactors. Extensive research on optically thin, high density outdoor mass cultures in flat plate photobioreactors by Dr. Hu and colleagues has demonstrated that for such cultures the smaller the light path the higher the productivity. Based on this work it is possible to project as much as 100 tC/ha-yr produced by such systems.

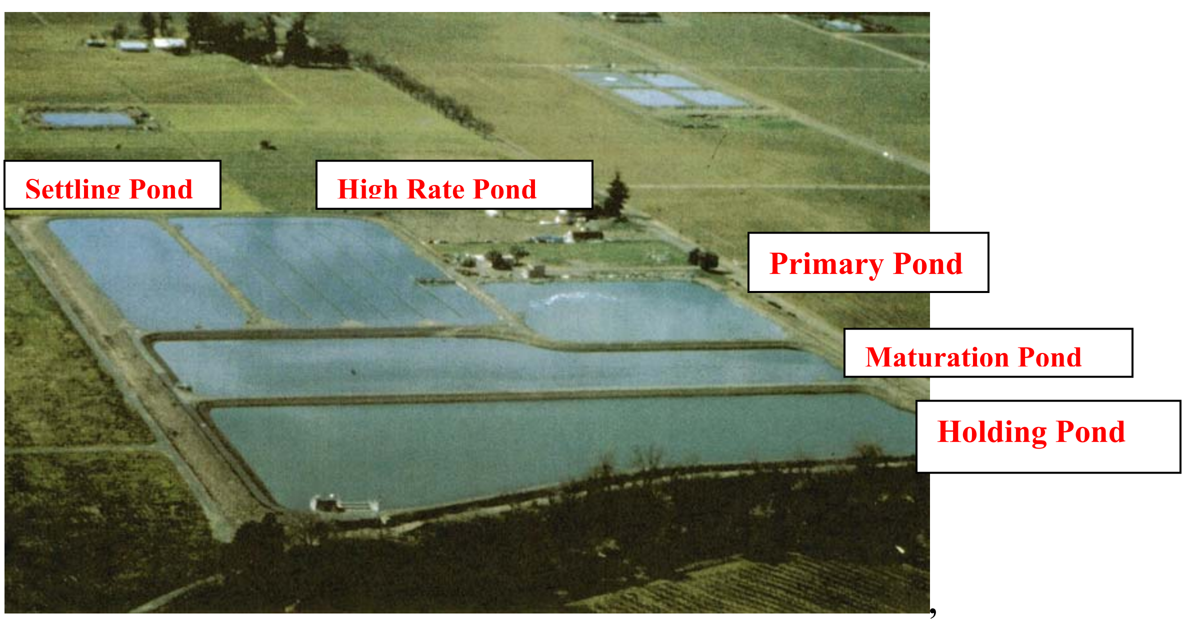
The fundamental issue in the production of algal biomass for greenhouse gas abatement is one of choice of mass culture device: open vs. closed system. Current experience with open systems suggests that for 0.5 ha ponds commercial production, such as of *Spirulina* and *Dunaliella*, is only 5 to 15 tC/ha-yr (corresponding to about 12 to 36 t biomass/ha per year, the latter being the stated production rates for companies such as Earthrise Farm and Cyanotech Corp.). There is a lack of data and R&D for larger scale system, e.g. 1, 10 or even 100 hectare ponds, as have been proposed by some techno-economic analyses.

For closed photobioreactors there is an almost complete lack of information on pilot-scale, let alone full-scale commercial operations, as few have operated, most of those suffered from significant design limitations, and data is not available for such commercial systems. Thus, at present the comparison of open and closed systems must be based on extrapolations from small-scale units and photobioreactor development is a major R&D need.

5. Bailey Green, Lawrence Berkeley Laboratory and Oswald Green LLP, California. "The Advanced Wastewater Ponds Systems (AIWPS) ® and Greenhouse Gas Abatement."

Advanced Integrated Wastewater Ponds Systems (AIWPS) ® is the technology developed over the past fifty years of applied engineering research led by Professor William J. Oswald at the University of California, Berkeley. The AIWPS® technology involves a series of ponds that carry out a treatment processes equivalent to conventional (such as activated sludge) wastewater treatment plants: primary ponds for sedimentation and methane fermentations of suspended solids; secondary, mixed raceway ("high rate") ponds for photosynthetic oxygenation of the remaining BOD (biological oxygen demand); settling ponds for the removal of the algal biomass produced in the high rate ponds, and final polishing ponds. AIWPS® facilities are less expensive to build and operate than conventional systems and provide a high degree of treatment, including natural disinfection of waterborne pathogens and parasites, in addition to reclaimed water and a the algal biomass, that can be used to produce methane gas and fertilizers. These systems abate emissions of greenhouse gases because they require much lower energy inputs than conventional processes and because of the methane that can be collected (using submerged gas collectors) from the primary ponds and generated from the algal biomass produced by such systems.

An example of such an AIWPS® facility is in St. Helena, in Napa County, California (visited by the participants of this Meeting on Sunday, April 27th, see below, operating for already 40 years and incorporates the essential elements of such a process: the influent facultative oxidation pond with deep sumps for settling of solids and anaerobic fermentations, the channelized high rate pond (to be retrofitted with paddlewheels in the near future); the settling pond that remove algal solids; the maturation pond that produces the treated water, which is applied to the grape orchard next to the property, with the final pond holding the irrigation water. This system is being upgraded with a dissolved air floatation unit to improve the algal solids recovery. Such facilities are now operating in several communities in California and other countries and this technology could make an important contribution to global greenhouse gas abatement. A proposal to develop and demonstrate such a process as part of the Network is being developed.



6. David Brune, Clemson University, South Carolina, USA, "The Controlled Eutrophication Process (CEP): Nutrient Recovery and Greenhouse Gas Abatement at the Salton Sea, California"

A potential site for a large-scale integrated microalgae CO_2 biofixation and greenhouse gas abatement process is the Salton Sea in Southern California. This large (900 km^2), shallow, inland lake receives about 10,000 tons of nitrogen and phosphorous (appx 10:1 ratios) per year from somewhat over 1 billion m^3 of agricultural drainage waters and other sources, resulting in massive algal blooms, fish kills and other negative environmental impacts. In addition salinity and water management issues will likely require over the next decade a large reduction in the size of the Salton Sea, and the management of salinity, plausibly through evaporative ponds. The use microalgae for nutrient removal from agricultural drainage waters in conjunction with salinity management, was already suggested and researched over thirty years ago, for applications foreseen for the turn of the Century. That time is now here.

The "Partitioned Aquaculture System" (PAS), developed at Clemson University, integrates microalgae biofixation of CO_2 with fish aquaculture, promoting a high rate of fish production and conversion of much of the algal biomass into rapidly settling flocculant solids that can be readily harvested by sedimentation. An adaptation of the PAS, the "Controlled Eutrophication Process" (CEP), is currently being evaluated by Kent SeaTech Corp in cooperation with Clemson University, in two ¾-acre (3,000 m^2) ponds, to obtain field data quantifying and demonstrating algal and fish productivities, algal harvestability, and waste N and, in particular, P recovery (as P is the main nutrient implicated in eutrophication). Based on projections from the PAS work of a productivity of 12 g C/m^2/day, this process would require about 1,600 hectares of algal ponds, recovering about 50 t algal biomass/ha-yr. In our present concept, the algal sludge recovered from the ponds would be blended with about an equal amount of waste biomass, such as waste paper, and fermented to methane gas, fueling 20 MWe of electricity generation, which would also provide the CO_2 required for this operation. The system would also generate some 20,000 tons of fish biomass (fresh weight) and about 7,000 tons of biofertilizers (on a N and P content basis). Based on energy production and fertilizer recovery from the algal biomass, as much as 200,000 tons of greenhouse gases would be abated in comparison with coal-fired electricity, a major source in this area, and the other benefits of the process, including nutrient recovery and recycling. A more rigorous analysis is required, but the economics of such a process would mainly depend on the value provided by the fertilizer removal process, at present uncertain. Also the value of the fish co-product is difficult to estimate, as its quality and markets remain to be defined. Assuming no cost for the waste biomass used to augment the anaerobic digestion process, doubling the gas yield for the algal-fish waste solids, the value of the biogas would be \$8 million, or \$5,000/ha-yr, at \$5/GJ (or MMBTU) for methane, and twice this for electricity at \$0.1/kWhr. For a system that would cost about \$100,000/ha in capital costs, without fuel or power generation equipment included, these returns can only cover a fraction of total process costs, requiring this environmental remediation function of this process as well as the fish co-product. Ongoing research at this site will demonstrate the feasibility of this process, which is based on fish-mediated algal harvesting. The key R&D challenge is to integrate the various processes – nutrient removal, algal harvesting, fish production, fertilizer recycling and energy generation into a single cost-effective process. A PAS/CEP process could be applied at other sites, such as from concentrated animal feeding operations and other agricultural drainage areas, providing for significant greenhouse gas abatement potential.

7. Michael Huesemann, Pacific Northwest National Laboratory, Sequim, Washington, USA.
Selection of Microalgal Species that Maximize Biofixation of CO_2 from Power Plan Flue Gases*

The long-term research objective of this project is to screen and select marine microalgae species that exhibit both high maximum growth rates and also have a high potential for CO_2 biofixation (high productivity) in outdoor mass cultures. Such strains are required to produce both the large amounts of inoculum required for the mass culture of algae, as well as allow for high productivity during the production stage. The basic hypothesis being tested is whether maximum specific growth rates (umax) are correlated positively or negatively with maximum productivity (null hypothesis: no correlation). The algae are inoculated into 750 1 liter Roux bottles under relatively low lights (about 200 uE/m2/sec) with fluorescent lights, with CO_2 in air (1.5%) in air and incubated initially for a few days to allow for batch growth, following which a period of daily dilutions is employed to determine productivity at various dilution rates. Several species of green algae (*Chlorella, Nannochloris, Chlorococcum*) and diatoms (*Chaetoceros, Thalassiosira* and *Phaedactylum*) have been successfully cultured in this set-up (Figure 1).

Figure 1. Semi-Continuous Photo-Bioreactor Setup

During batch culture growth the main method of analysis is Optical Density, while during semi-continuous growth both OD and ash-free dry weights are used. Productivity is calculated from dilution rates times cell densities at dilution time. For the seven strains tested in this experimental protocol, there were large differences in productivity, ranging from 200 mg/liter-day to 1,200 mg/liter-day. Maximum growth rates ranged from half to two doublings per day. There was no apparent correlation between growth rates and productivity, but the sample size was too small to allow any final conclusions. Also, the maximal growth rates under these conditions were well below maximal doubling rates observed with similar strains in laboratory cultures, and, the reasons for this remain to be investigated. A mathematical model of growth rate and productivity is under development and the P vs I curves (photosynthesis rate as measured by O_2 electrode) and light intensity relationships are being explored.

This work is ongoing with new strains that are suitable for outdoor mass culture.

8. Joseph C. Weissman, SeaAg, Inc., Vero Beach Florida, USA* " Comparison of Marine Microalgae Culture Systems for Fuels Production and Carbon Sequestration"

Microalgae technologies for the production of renewable fuels and the mitigation of greenhouse gases are being developed in the U.S. and abroad using both closed photobioreactors of various designs and open ponds. A direct experimental comparison of the attributes of these systems, in particular in regards to their relative CO_2 biofixation potential, that is productivity, is required. Three photobioreactor designs were compared for usefulness as mass culture systems for large-scale microalgal production: open, raceway ponds; thin vertical flat plates; and deep, clear-sided, vertical tanks. The first is the only system currently used commercially for large-scale applications. The second is of a general type often proposed as a potential alternative. The third type is used mainly for small-scale aquaculture feed applications. The objective of the study was to compare these photobioreactors on basis of the practicality of their operations, the biomass productivity actually achieved, and the potential for increasing productivity, as determined in experiments comparing solar energy conversion efficiencies in ponds with reduced light input.

Experiments were carried out with a marine diatom, *Cyclotella sp*., initially with two ponds and several vertical photobioreactors operated in parallel under identical conditions to determine the reproducibility of the experimental results, which was very good. The vertical photobioreactor systems were not as stable, often crashing and thus precluding long term data collection. This may have been due to the aeration -mixing system. There was little difference in productivity at three different pH's, 7, 8, and 9, suggesting that pH (a direct measure also of dissolved CO_2 in solution and available to algae) was not a critical factor in these experiments.

As expected, the biomass production rate per unit area of light interception area was greatest for the open ponds, but photosynthetic efficiency was lowest. In ponds, all of the surface area receives high intensity light while the direct rays of the sun are spread out over the surface of vertically oriented systems. Thus in the latter the cultures see a lower light intensity which is more favorable for algal productivity, if calculated on a per unit light input or absorbed. Experiments were performed to determine if open ponds could be more efficient in the use of incoming photons. In one set of experiments 70% of the pond surface was covered with white plastic board, arranged to alternate illuminated dark and lighted areas. With only one fourth of the surface receiving light (photons), biomass productivity was still 50% of the control pond with a completely uncovered surface. Thus photosynthetic efficiency was twice as high. Similar results were obtained with shade cloth that reduces overall light intensity by a similar amount over the entire pond. These and other experimental results suggested that both light saturation and photoinhibition significantly reduce algal productivity in mass culture ponds. The approach to maximize productivity cannot rest on vertical photobioreactors as these suffer from many limitations, in particular the economics of such systems. A research plan was devised to minimize the adverse effects of both light saturation and photoinhibition by using algal strains that are selected to have reduced harvesting pigment content (see presentation by Dr. Polle below)**. Such algal strains could overcome current limitation and exhibit productivities exceeding 100 metric tons dry organic biomass per hectare per year, a key goal of this Network.

*Supported by a U.S. Dept. of Energy Small Business Innovative Research (SBIR) Phase I Grant, #DEFG002ER83515. **Note added after Meeting: in June SeaAg, Inc., received a 2-year, $600,000 Phase II SBIR project to carry out this research in collaboration with Dr. Juergen Polle.

9. Juergen Polle, Brooklyn College, Brooklyn, New York, USA. “Chlorophyll Antenna Size of the Photosystems and the Capacity of Photosynthesis in Green Algae”

Dense microalgal mass cultures operating outdoors under full sunlight exhibit much lower productivities than projected based on laboratory data obtained at low light intensities, the so-called “light saturation effect”. This is because, an algal cell in a dense, mixed pond experiences on average very low light intensities. In response, the alga assembles large numbers of chlorophyll (Chl) molecules, typically 200 or more, into pigment arrays, the so-called "antenna” or “light harvesting” chlorophylls, which allow it to capture as many photons as possible. This results in the algal cells that find themselves at the surface of the pond capturing many more photons than can be processed by the photosynthetic machinery, with these excess photons degraded as heat or fluorescence. Overall, the wastage of photons available for photosynthesis can be as high as about 70 - 80% of the total photons captured by the algal culture, thus reducing solar conversion efficiencies to levels far below their potential. Furthermore, such excessive but ineffective photon absorption by the antenna chlorophyll results in damage to the photosynthetic apparatus (photoinhibition), further reducing solar conversion efficiencies and productivities.

It was speculated already several decades ago that microalgae with a smaller number of Chl antenna molecules would not be subject to wasteful dissipation of excitation energy and reduce the shading of cells lower in the photic zone, resulting in much higher solar conversion efficiencies and productivities by algal mass culture. However, a search for such strains in nature did not reveal any – as was expected from the strong selection pressure that would be exerted on individual cells to maximize photon absorption in light limited environments. With the advent of modern molecular biology and genetic engineering techniques, it has now become possible to develop mutants with reduced, that is truncated, Chl antenna sizes. This was applied first to a model organism, *Chlamydomonas reinhardtii*, which much genetic information is available. Some work was also carried out with *Dunaliella salina*, an algal species already mass cultured.

The Chl antenna sizes of the two photosystems and analysis of their rates of photosynthesis (photosynthetic efficiency and capacity) can be measured spectrophotometrically and with oxygen electrodes, respectively. Experiments were performed with *C. reinhardtii* using a wild type, a Chl b-less, a Chl-deficient, and a carotenoid deficient mutant. Under low light conditions, in the mutants the Chl antenna size for PSII was reduced by up to 50% of that of the wild type level. For PSI the Chl antenna size in the mutants was maximally reduced by only 20% as compared to the wild type. Similar results were obtained with the wild type grown under low light and high light as well as for a low-light grown Chl-deficient mutant of the unicellular green alga *Dunaliella salina*.

In summary, it has been shown that it is possible to obtain mutants with permanently truncated Chl antenna sizes of both photosystems and that these exhibit increased rates of photosynthesis at high light intensities. However, several challenges still are apparent: the Chl antenna size of PSII appears to be more readily reduced than that of PSI and reduction in PSII antenna size has not yet approached the maximum thought to be possible. It is predicted that truncation of the Chl antenna size of PSII alone to its possible minimum would result in a 2.5 fold increase in the photosynthetic efficiency of microalgal cultures under full sunlight intensities. However, current increases fall far short of this goal and continuing R&D is required in this area.

10. Patrick Hallenbeck, Universite de Montreal, Monteral, Quebec, Canada. "Biological Hydrogen Production by Microalgae: Fundamentals and Processes"

Biological hydrogen production can be accomplished by three types of enzymes: nitrogenase (turnover number 6.4 s-1), Ni-Fe hydrogenase (evolution type, turnover number 98 s-1), and Fe hydrogenase (turnover number 6000-9000 s^{-1}). When also considering the differences in molecular weights and requirement for ATP by nitrogenase (4 ATP /H_2), it is clear that nitrogenase-based systems are not plausible for practical applications and Fe –hydrogenase based processes would be the preferable option, all else being equal. Of course, the O_2 sensitivity of the hydrogenase enzymes themselves and even greater of the reaction they catalyze, makes it imperative to work under anaerobic conditions. The feasibility of developing a sufficiently O_2 resistant hydrogenase reaction to allow so-called "direct biophotolysis" (photosynthetic water splitting without intermediate CO_2 fixation) is presently speculative. Under anaerobic conditions, hydrogenase generally does not seem to be a limiting factor, the more critical issue generally is the supply of the low redox potential reductant required by H_2ases.

For any H_2 production by microalgae, processes generally termed "biophotolysis", the key issue is the efficiency of the conversion of solar energy into hydrogen. A 10% conversion efficiency is often given as the maximum possible and as the goal of direct biophotolysis R&D. For the sunnier regions of the U.S. receiving 5 kWhr/m^2/day of sunshine, this translates to \$10/m^2-year assuming a value for the H2 of \$15/GJ (a goal of the U.S. DoE Hydrogen Program). The key issue is the cost of photobioreactors that must capture and contain the H_2, with \$100/m^2 generally considered a minimum for such systems, even when projecting large scales and low costs. Thus, even with a very favorable solar efficiency assumptions any process using photobioreactors to cover the entire light collection area, such as direct biophotolysis processes or heterocystous cyanobacterial systems and other single-stage concepts, would be economically impractical, even if technically feasible. Technical feasibility remains to be established for such processes.

"Indirect biophotolysis" involves concepts in which CO_2 fixation into a fermentable substrate (e.g. starch, in green algae) takes place either temporally or physically separated from the H_2 production reactions. Indirect processes have the dual advantage that the H_2ase reaction is separated from O_2 and that the main solar conversion process, the CO_2 fixation stage, could take place in open algal ponds such as proposed for Network projects and typically having capital costs of about \$10/m^2. H_2 production thus becomes a fuel conversion option, similar to methane fermentations of algal biomass. Two options are available for the H_2 production stage: a light-driven process or a dark anaerobic fermentation. Light reactions have the advantage of allowing, in principle, a stoichiometric conversion of starch (or similar C-storage polymers) to H_2. However, these require photobioreactors, with the key issue the solar conversion efficiency of such a process - photons per H_2 produced. In any photobiological process a minimum of one photon per electron transfer is plausibly required, or two photons per H_2. This amounts to near half the theoretical conversion efficiency of a direct biophotolysis process. In short, the photobioreactors would still need to be quite large, and thus even indirect processes are too expensive. The best option for H_2 production using microalgae is to use dark fermentations, preferably using their endogenous hydrogenases, found in cyanobacteria and green algae, as these would allow more efficient utilization of intracellular reserves than bacterial fermentations. Microalgae could provide an additional future biomass resource base for high-yielding H_2 fermentations. Research on this topic is suggested as a promising R&D topic for the Network.

C. TECHNICAL DISCUSSIONS

1. Summary Descriptions of Ongoing Projects by Network Members.

The following briefly summarizes the currently ongoing projects carried out or supported by Member Organizations of the Network and the collaborations and cooperation between projects.

a. Arizona Public Services (Qiang Hu, Milton Sommerfeld, Dan Musgrave and **Duane Pilcher)** This project, being carried out by Arizona State University and Universal Entech LLP is aimed at achieving a synergy between nutrient recycling and energy production with microalgae. The project focuses on wastewaters from animal feed lots and other agricultural wastes and is using tubular photobioreactors of up to 10,000 liters. One objective is to establish testing protocols to allow comparison ("apples to apples") of such a process with other processes to recycle nutrients from animal wastes. The APS budget is $170,000, with R&D currently at the laboratory stage and scale-up work is anticipated later this year. (See presentation by Dr. Hu)

b. U.S. Department of Energy – Pacific Northwest National Laboratory (Dr. Michael Huesemann, see Presentation). This project, studies the relationship between growth rate and productivity and is funded at a level of $180,000 per year for three years, with the project about half way through this period. This project will use the same strains as the next described projects.

c. U.S. Department of Energy- SBIR Program (Joseph C. Weissman, Juergen Polle, see presentations) U.S. DOE under the SBIR program has funded a Phase I project for $100,000 and a Phase II proposal for $600,000 (approved in June, 2003), to use mutants developed by Dr. Polle is pending. This project is being carried out in collaboration to the previously listed one.

d. EniTecnologie (**Dr. Paola Pedroni**, see pesentation). This project is currently funded for about $500,000 for the first year of a four year planned effort. Initial work is comparing outdoor open ponds and photobioreactors comparing marine species, initially *Phaeodactylum*, and *Tetraselmis*. This project is being carried out with assistance of Prof. Mario Tredici of the U. of Florence and in coordination (e.g. strain and data sharing) with the DOE funded projects.

e. Rio Tinto (**Dr. David Barr**). Three projects are ongoing, two in Australia (at somewhat over $200,000) for work with RuBisCo and for CO2 mineralization by microalgae, and one project on microalgal RuBisCo carried out by Maxygen and Codexis (> $1 million). (See Presentation).

f. ExxonMobil (Roger Prince). (See Presentation). The project on *Botryococcus braunii,* funded at the University of California, is being discontinued, along with membership in the Network, due to the shift of the ExxonMobil R&D efforts in the area of greenhouse gas abatement technologies to Stanford University. Stanford University is presently investigating microalgae systems for H_2 production with both direct and indirect biophotolysis systems being pursued.

g. ENEL Produzione Ricerca is carrying out laboratory R&D on microalgae production using photobioreactors, with assistance of Prof. Mario Tredici. The absence of the ENEL Steering Committee representative did not allow for an update on this project, its budget or the company's and research team's future plans. (See Minutes of the Kyoto Meeting for a brief summary).

2. Summaries of other Ongoing and Proposed Projects Related to the Network.

a. Salton Sea Project. This ongoing project (see above) kby Kent SeaTech Corp. with Dr. Massingill as P.I., funded by the Salton Sea Authority, about $250,000 for the first year, with a second year proposal pending for a similar amount of funding. A proposal was submitted to USDOE for work on this process by Kent SeaTech and Clemson University. (Note added in June: this proposal was not funded but is planned to be re-submitted). A related proposal ("The Microalgae Platform") was submitted to a USDA-DOE joint solicitation by Clemson University with Kent SeaTech and John Benemann. Other sources of funding are being explored. The main objective of the Salton Sea Project is to move to a 40 acre demonstration project in 2 – 3 years, possible by focusing on high value co-products, specifically fish. Prof. Brune has a proposal pending with Joseph Weissman for clean-up of P from influents to Lake Okechobee, Florida.

b. Biofertilizer Project. A proposal was submitted by SeaAg, Inc. (Joe Weissman P.I.), with participation of Cyanotech Corp. (for scale-up) and Clemson University (to carry out anaerobic digestion studies) to the U.S. Dept. of Energy and (under an SBIR solicitiation) and to the National Science foundation, on organic biofertilizer production with N_2-fixing algae. This proposal was not funded by either agency but will be resubmitted later this year, possibly to other funding agencies. Biofertilizer production is one area emphasized by the Roadmap.

c. Sunnyvale Wastewater Treatment Plant. This project is in early stages of discussion with the Sunnyvale Wastewater Treatment Plant. The objective is to develop an integrated process using high rate ponds to improve effluent quality (mainly reduce nutrients discharged into the Bay) as well as provide additional biomass for anaerobic digestion to supplement biogas current produced by their anaerobic digesters and the depleting landfill gas supply. A proposal is being developed by John Benemann in collaboration with Lawrence Berkeley Laboratory (Dr. Bailey Green) and, possibly, with participation of other Network members (e.g. GTI and EPRI). A proposal is planned to be written and submitted this year to the U.S. Department of Energy.

d. Microalgae Projects in India. Dr. Pradeep Dadhich listed four proposals from the TERI team on microalgae technologies that could be carried out in relation to the Network in India: Water treatment, anaerobic digestion of microalgae, screening for suitable strains of microalgae and establishment of a microalgae culture collection. These projects could be carried out in collaboration with Network projects and members.

e. *Botryococcus braunii* Proposal. Dr. Juergen Polle, in collaboration with Dr. Joseph Weissman (SeaAg, Inc. and with participation of Dr. Michael Huesemann (PNNL) and Roger Prince (ExxonMobil) have prepared a proposal on the mass culture of *B. braunii* for hydrocarbon production. The plan for this proposal was developed during this Network meting.

f. Biohydrogen. John Benemann, Network manager, is submitting a proposal to U.S. DOE, with the University of Hawaii and others to develop H_2 production technologies by dark fermentations. One of the project tasks will be to investigate H_2 production using the endogenous hydrogenases and starch or glycogen reserves of microalgae in dark fermentations. This project would fit into the Network objectives, presenting an alternative fuel conversion option.

3. Discussion of R&D Issues.

Although the technical discussions were to be structured along several specific topics (e.g.: potential for greenhouse gas abatement with microalgae; biotechnology and strain isolation and improvement; engineering and economics, biofuels and co-products, etc.) in the event the technical discussions were free ranging.

a. Conversion Processes. The use of algae biomass as a fossil fuel replacement requires its conversion to fuels. Gasification and other thermochemical processes are not applicable, due to the high moisture and nutrient content. Fermentations are the only plausible way, with methane fermentations suitable for microalgae biomass, but overall yields are generally not high. This is an area requiring some applied R&D. Dan Musgrave mentioned that Universal Entech LLC is carrying out such a study as part of the Arizona Public Services funded project. It would be desirable to obtain high starch content algal biomass more suitable for ethanol or even H_2 fermentations. Nutrients, typically N, limitation, are often used to achieve such a composition change, with the key issue being is how to obtain high levels of such storage products without loss of productivity. This appears to be feasible, in the view of John Benemann.

b. Harvesting. This is a perennial issue in microalgae biotechnology. One preferred process, as discussed in the Technology Roadmap, is bioflocculation, the often observed phenomenon in which algal cultures, after being removed from the paddle wheel mixed pond, flocculate and settle, allowing recovery of a concentrated biomass. Dr. Brune (Clemson University) described how fish, specifically Tilapia, can be used to harvest microalgae by converting a large fraction of the algal biomass into rapidly settling biomass. A greater understanding of either process should allow its application in a more controlled and effective manner. At wastewater treatment plants, such as Sunnyvale and St Helena (both plants visited by meeting participants) dissolved air floatation is used to harvest chemically flocculated algae solids. Although expensive this process can be used until bioflocculation is perfected and if lower cost technologies are not be applicable.

c. Productivity. Of course, the greatest challenge remains to produce the greatest amount of biomass possible within economic constraints. The near term next five years, of the Network are the achievement of 100 t/ha-yr, demonstrated with outdoor ponds on a year-round basis at a favorable site in the U.S. How to reach this goal was discussed in several presentations by David Barr (Rio Tinto), Juergen Polle (Brooklyn College), Qiang Hu (Arizona State University) and Joseph Weissman (SeaAg, Inc.) and need no repeating here. It can be noted however that the 100 t/ha-yr goal of the Network is only an initial, even minimal one. Productivities of 150 to 200 t/ha-yr may be feasible in the longer term. The maximum practically achievable productivities are not know but are thought to be in this range, perhaps even somewhat higher, based on theory, laboratory data, and optimistic extrapolations of such information to outdoor systems assuming no light saturation, no photoinhibition and no major restrictions in CO_2 assimilation (e.g. RuBisCo) or respiration. Achieving these productivity objectives requires both genetic approaches as well as of methods for scaling up algal cultures in open ponds. For such purposes an inoculum production process will be required. In the longer-term it may be possible to combine various productivity improvements (e.g. in light saturation, photoinhibition, CO_2 fixation and respiration) into single strains.

d. Genetics. Recent developments in genetic systems for microalgae were discussed in some depth by Paul Roessler (Dow Chemical Co.). The *Chlamydomonas* genome is being completed, and the genomes of several other microalgae, specifically *Phaeodactylum* and *Thalassiosira*, will not be far behind, with genomic information advancing very rapidly now. Gene chips will become available to help find genes that operate in different environments and this may shed some light on the genetic factors that allow some algae to dominate the ponds or specific environments better than others. These "tool boxes" will greatly simplify R&D in this field. Several new genetic systems are now available that allow for microalgae genetic and metabolic engineering. This combination of rapidly expanding genomic databases, our ability to utilize the same, and the availability of genetic systems, is of great significance in the future development of the Network, most immediately in ongoing R&D in antenna chlorophyll reduction and improvements in RuBisCo.

e. Antenna Size Reduction. Dr. Juergen Polle (Brooklyn College) pointed out that by combining classical mutational approaches with advanced molecular biology tools, now allows the development of improved, but not genetically modified, mutants that exhibit increased photosynthetic activities through antenna truncation. Dr. Weissman presentation suggested that indeed antenna truncation should greatly increase productivity, and achieve in horizontal bioreactors (e.g. ponds) the productivities projected for optimally spaced vertical closed photobioreactors. Developing such mutants and demonstrating their enhanced productivity in outdoor systems is a key R&D goal for the Network.

f. Photobioreactors and Inoculum Production. Both open ponds and closed photobioreactors have roles in the development of a microalgae technology, the latter through the production of the inoculum required for large-scale cultivation of the specific, even genetically improved, strains to be generated in the future. Dr. Paola Pedroni discussed the EniTecnologie project where both types of algal culture systems will be tested on a side-by-side comparison basis (see presentation). For inoculum production, whether of wild type strains or mutants, the strains will be required to grow quickly, while for biomass production high productivity is more important than fast growth (maximal growth rate). The relationship between these attributes in microalgae is being investigated by Dr. Michael Huesemann (LLNL) (see presentation). This issue is at the core of the technology of algal mass culture. The issues related to productivities of high density algal cultures in photobioreactors, relative to open pond reactors, were also discussed, by Drs. Hu and Weissman. The main issue, however, with closed photobioreactors, is not productivity but economics, that is cost per unit area, both capital and operating. For low value products only simple open ponds can be used. High value products, which would allow use of more expensive systems, would not have any significant market sizes, at least in the context of CO_2 abatement. They would, however, be required for inoculum production.

g. Unialgal and Mixed Cultures. A fundamental issue is the choice between monocultures and mixed cultures (or even polycultures, such as algae and fish). For monocultures, it is likely that higher value co-products would be required, as opposed to wastewater treatment alone, which could more readily accommodate mixed cultures, as pointed out by David Brune. Still even in wastewater treatment, in the long run utilization of specific algal strains would be desirable. The aim of the Technology Roadmap is to develop and demonstrate technology that allows large-scale production of genetically selected and improved unialgal cultures.

h. CO_2 Capture and Utilization. Co-location of algal systems and power generation units are required for algal biomass production to supply the CO_2. This brings into focus the issue of CO_2 capture by such systems, which requires flue gas handling, collection, and distribution systems, as well as CO_2 transfer into the ponds. Although not considered major impediments to the development of this technology, these require more engineering design and cost analysis. The power plant operating at Cyanotech, discussed briefly by Dr. Cysewski (Cyanotech, Inc.), is an example of such a system, although the CO_2 capture system would be quite different for other algae. Flue gas CO_2 capture and utilization will be a significant factor in overall costs.

i. Engineering Studies. The scale of the systems envisioned by the Technology Roadmap are large, an order of magnitude, even two, larger than the currently existing commercial systems. Although of very different scales and objectives, the fundamental design remains similar. One need is to study the hydraulics of very large, unlined, paddle wheel mixed, raceway ponds, to determine the optimal largest single pond size. Earthrise Farms, Inc. (Amha Belay) has operated two large single ponds of several acres in size, without plastic liner and a single paddlewheel. A 5 hectare single pond with multiple channels is being operated in Hollister, California. Bailey Green (LBNL) showed the such ponds in wastewater treatment, specifically at the relatively new Delhi, California, plant. Cost estimates for large-scale processes are available from prior work, but need to be updated.

j. Systems Analyses. The processes envisioned by the Roadmap still require considerable development. One near-term need, pointed out by several member representatives (e.g. Vipul Srivastava, GTI, and David Barr, Rio Tinto) as well as by other meeting participants (Dan Musgrove, Universal EnTech, and Blaine Metting, PNNL), is for a "Life Cycle Analysis" of these microalgal systems, which would move beyond the purely economic or engineering to address such issues as sustainability, externalities, whole C accounting, and resource needs, in particular of water, and of product outputs, including reclaimed water and energy. Paul Roessler pointed out the need to compare water use efficiency (WUE) of algal ponds and irrigated crops. Duane Pilcher (Arizona Public Services) pointed out that for APS, the main objective, in the long-range, is to obtain a "C-credit" from the CO_2 recovery process. David Barr suggested that for the process to work economically the C-credit would be at most a "bonus", with higher value co-products or processes providing the bulk of the operating and capital costs.

k. Conclusions and Action Items. The above provides a brief overview of the topics addressed. One major conclusion is that there is no single major obstacle to the development of this technology, however there are many significant ones. Overall the consensus was that demonstrating significant productivity increases should be a central focus of the Network R&D activities. The most immediate "action item" for immediate work by the Network, is the last one (j.): "Systems Analyses". The Network Manager will present at the next Network Technical Meeting in Paris a "Study Plan" for a "Systems and Life Cycle Analysis" to be carried out during this year. This report would cover the above listed issues: greenhouse gas abatement, energy balances, C-balances, water use and efficiency, other resources consumption, externalities, sustainability, and overall significance to regional and global greenhouse gas reduction needs and goals over the current century.

D. DISCUSSION OF NETWORK ISSUES.

1. Membership.

a. Need for new Members. The Network would benefit from additional members. Although the eight members recruited for the first year were well above the minimum envisioned as needed to initiate the Network, membership is still small and in several cases has not yet committed to carrying out particular R&D activities in this field. Further, the resignation of ExxonMobil, even though for reasons extraneous to the Network, also suggests that even staying at the present small membership level requires additional recruitment. This was tasked as one of the main activities for the Network Manager, John Benemann, over the next year. He reported that new membership, and retention of existing membership, was indeed a major priority.

b. Participation by U.S. DOE. One issue over the past year had been the relative lack of participation by the U.S. DOE in the Network meetings, and indeed the uncertainty of its future role in the Network. The support of U.S.DOE was critical and substantial in both the formative stages of the Network and over the past year in supporting the preparation of the Roadmap by the Network Manager. Thus the Network has greatly benefited from the U.S. DOE leadership in helping to organize and continuing to support the Network. The specific projects currently funded by U.S. DOE and related to the objectives of the Network (those of Michael Huesemann, PNNL, and Joseph C. Weissman, SeaAg, Inc., see above) are supporting projects that are at the core of the Network R&D goals. This has overcome some of the uncertainties of the last year. The Network manager will work to enhance the support of specific projects with a more active participation by the U.S. DOE, both directly and through the National Laboratories, specifically PNNL. Dr. Blaine Metting indicated that PNNL is currently evaluating joining the Network.

c. International Membership. The Network has a limited international membership, with four of (now) seven Member being U.S. organizations. Thus an objective should be to expand the membership internationally. John Benemann indicated that this would be the main objective of the next Network meeting, to be held in Paris September 29th, in conjunction with the next ExCom meeting of the IEA Greenhouse Gas R&D Programme. Some interest had been expressed by the some ExCom members (including those that toured the Sunnyvale Plant, see tour report) and this will be followed up. India has expressed interest in the Network, and a mechanism will try to be developed for India to be able to join at least on an associated basis, without need to pay full membership fees. As reported by Yoshi Ikuta, Chugoko Electric in Japan is mentioned as a future participant if they go ahead with their microalgae project past this initial exploratory phase. Endesa, in Spain, an observer to the meeting in Almeria is contemplating a project in microalgae. These and other leads will need to be followed up. Now that the Network is an ongoing concern, it will likely be easier to recruit new members, based on their interest in microalgae technologies for greenhouse gas abatement.

d. Small Businesses. One issue raised was how smaller organizations, including not-for profits and private companies, could participate in the Network. Options and mechanisms for such participation, including possibly a reduced annual fee, will be developed for the next Network meeting and discussed at that time.

2. Technical Advisers.

With the Roadmap completed, the consensus of the Members was that the Technical Advisers could best serve on an ad hoc basis, depending on specific needs, to allow focusing of resources on specific tasks, such as the planned "Systems Analysis" (see below). Also, it would be desirable to expand the Technical Advisers pool. The Network Manager agreed that with the completion of the Technology Roadmap the role of the Technical Advisers could now change. Several Technical Advisers are involved in some aspect or another of the R&D, from funded projects to submitted proposals, and their participation is assured from that perspective alone. He suggested that the attendance of Technical Advisers of future meetings could be individually targeted depending on venue, agenda, R&D issues and other factors. However, he also opined that a once a year meeting by all participants would be beneficial. John Benemann expressed his deep gratitude to the Technical Advisers for their enthusiastic participation in the Network and their many inputs during the technical meetings, during development of the Technology Roadmap, and on many other occasions over the past year and before. He recommended that the present Technical Advisers remain in that position for the coming year. He also recommended increasing the pool of Technical Advisers and nominated Dr. Bailey Green as a new Technical Adviser. He also thanked the Invited Researchers, who participated in the meeting and looked forward to their future participation in the Network through their ongoing or proposed projects.

3. Meetings.

During the first year of the Network three meetings were held. For the coming year only two are planned. The first meeting (4th Network Technical Meeting) is being held in September 29th in Paris, France, and will further strengthen R&D collaborations and introduce the Network to potential new members, including interested representatives at the IEA ExCom Meeting. The Agenda for this meeting will include the herein listed "action items" (Section D), specifically the proposed "Systems and Life Cycle Analysis" report, and the issues addressed in the present and the following Sections. The meeting will present recent R&D activities by some Network participants, though the main objective will be to allow for discussions of matters of interest to the meeting attendees, technical and otherwise. A second meeting (5th Network Technical Meeting) will be held sometime prior to the end of the Network fiscal year second year and would be a more in depth review of technical progress. Suggestions for a venue are welcome.

4. Budget

Last year's budget of $72,000 has been fully expended. The budget for the present Network Fiscal Year, starting June 1st, depends on the number of members contributing to the Network. Also, the Network Manager will not be supported by the U.S. DOE this year, so his support from the Network will increase from 7 to 12 months ($28,000 to $48,000 per year at the same level of effort, based on nominally a quarter time). However, it is expected that the meeting costs will be much reduced from the first year, from about $40,000 to less than half this. I was decided to finalize a budget at the next meeting of the members (scheduled for September 29th, in Paris), by which time the overall resources would be clearer. In the meantime the Network would continue to operate with John Benemann as Network Manager at the present level of effort.

5. Benefits of Membership.

a. Benefits of the Network. One issue that arose was the need to better justify the Network in terms of the benefits it provides to the Member Organizations, and how they benefit specifically, compared to non-members who could obtain public information as it becomes available from Network activities and R&D projects. This section discusses these issues. Overall, the main benefit of the Network is in assisting Member Organizations to develop microalgae-based technologies, typically as part of a broader portfolio of GHG abatement technologies. Scientific and technological advances needed to demonstrate the practical feasibility of such microalgae biofixation of CO_2 for GHG abatement require extensive, long-term, integrated R&D efforts, both basic and applied. The multidisciplinary skills and many topics requiring R&D, both laboratory and outdoors, is not achievable by any single organization, but could be achieved through the Network. The Network is not a funding mechanism, but was structured to accommodate a diversity of interests, goals and stages of development among the Members.

b. Benefits for Member R&D Projects. The specific benefits of Network membership depend on the interests and goals of the Participating Organizations. For those with already ongoing R&D projects, the Network provides access to technical expertise and assistance, difficult to obtain on an individual basis, and a way to quickly advance to the cutting edge of R&D. The Network provides mechanisms to become part of an overall coordinated R&D effort, rather than stand-alone, isolated projects which have a lower probability of succeeding on their own. The main premise of the Network is that no single organization will be able to marshal, or justify, the resources required for technology development in this field. Benefits for member organizations that are not yet decided on specific R&D goals or projects in this area include the access to technical information that the Network provides, allowing them to develop projects best fitting their own interests and goals and to collaborate or participate in already ongoing projects.

c. Risk, Cost and Start-up Reductions for R&D Projects. The major value of participation in the Network is in terms of reducing the risks of the R&D project, which are substantial even in the best cases, and certainly high in the case of CO_2 abatement technologies generally. It also reduces the “start-up” or learning curve time for such a project. This is the major value of membership in the Network: it provides a mechanism for Member Organizations to reduce the cost and risk of their own in-house R&D projects in this area. It also provides a mechanism for participation in or support of extramural projects, in particular multiparty projects.

d. The Technology Roadmap. A major benefit of the Network is that the technical team - the Network Manager and Technical Advisers - have developed the Technology Roadmap, an R&D plan to achieve the goals of the Network: development within five years and demonstration and implementation within ten years of viable technologies for power plant flue gas CO_2 abatement. The Roadmap identifies specific R&D areas where technical progress can and should be made. The Roadmap allows for limited near-term applications and much for more ambitious goals for the future. Wastewater treatment and nutrient removal provide near- to mid-term applications that could have significant potential globally. Nutrient recycling and N_2 fixation by microalgae could become a source of relatively high value biofertilizers. Large volume and high value co-products would allow for additional applications, with integration of these processes, for example co-production of fish with nutrient removal at the Salton Sea, for example.

e. Technical Expertise. However, the Roadmap provides only a rather generic and general guide, and will become a public document when it is published, eventually, by the U.S. DoE. The benefit derives from the expertise of the Network Manager and Technical Advisers who can assist Member Organizations translate the Roadmap into specific R&D approaches and projects. Also, the Network provides for interaction with other R&D managers who have similar goals in greenhouse gas abatement and renewable energy technologies. The most important benefit of participation in the Network is that the members can be fully confident that they are receiving the best possible available technical insight and expertise in this field. The Network technical team has a diversity, breadth and length of specific experience that can not be replicated. It guarantees that Member Organizations can avoid the many pitfalls that have befallen prior and indeed still ongoing efforts in this field. And it provides ample opportunities for integrating specific R&D projects into an overall framework of collaborative and cooperative efforts with similar goals.

f. Selection of Projects Included in Network. The Network Manager, with the support of the Technical Advisers, has not included or invited a number of ongoing and funded projects in the U.S. and elsewhere that deal with microalgae power plant CO_2 abatement. That is because those projects are considered by this team to be technically unsound and without redeeming scientific or engineering value. Specifically, and sadly far from exclusively, in this context can be mentioned projects that use concentrating mirrors that transmit light into closed photobioreactors with optical fibers or light guides. Such approaches, first suggested over 25 years ago, were the focus of the large Japanese program of the 1990's, which spend hundreds of millions of dollars without significant advances or even technical accomplishments. A cursory search of the internet reveals in the U.S. an informal "network" of researchers in this specific field ("optical fiber photobioreactors") that perhaps rivals the present Network in participants and even resources. Regardless, the Network has ignored these R&D approaches and activities, as they are counterproductive to the goals of the Network. Avoiding the "far side" of microalgae R&D for greenhouse gas abatement would be by itself sufficient justification for membership.

g. Information Management. For full benefit from the Network, Member Organizations will need to establish information sharing as a major goal for these R&D projects. Of course, such sharing of information must be decided on a case-by-case basis, and some specific information would remain proprietary. However, considering the rather generic nature of this technology, this is perhaps not as major an issue as in other fields. An information management system must be developed and is a major goal for the Network this year (see next section).

h. Overall Benefits of Membership. It his hard to quantify the above listed specific benefits, and they will vary among members, with each needing to evaluate their own benefit of membership. The high costs and risks of any R&D projects, makes the risk reduction the Network provides a high return on investment. Ultimately, the benefit of the Network to the Member Organizations is the participation in and benefit from the development of this new, useful and significant technology for not only greenhouse gas abatement but also for renewable fuels and other resources, including reclaimed water, feeds and foods, commodities and environmental services. Further development, demonstration and communication of these benefits is a major objective for the present year, extending beyond information management. Finally, any benefits for non-member organizations resulting from the work of the Network, will greatly delayed and of limited value in the planning or execution of R&D projects in this field.

E. PLANNED ACTIVITIES OF THE NETWORK.

The following summarizes the specific and general activities planned for the Network during the present fiscal year (starting June 1, 2003), as already discussed in prior sections.

a. Systems and Life Cycle Analysis Report. Several members desired a greater emphasis on the economics of microalgae systems for greenhouse gas abatement, as well as "life cycle analysis" (LCA) of microalgae systems (See end of Section C). There was also interest in developing an assessment of the potential for microalgae to reduce greenhouse gases based on scenarios that incorporate the technological advances being foreseen from and by the Network. This should consider the geographical context – some countries cannot consider devoting large land areas to algal systems, others do not have suitable climate, water resources, etc. It would be important to demonstrate where such systems could be introduced. Applications to LDCs should consider the specific infrastructure requirements of such processes. The Network Manager, as part of his work, will update existing information in this area as well as carry out necessary new studies and analyses to develop a "Systems and Life Cycle Analysis" Report during the present year. A proposed report outline and study plan will be presented at the next meeting in Paris, on September 29th. It is envisioned that this will be a major activity of the Network this year.

b. Information Management and Network Website. The Network website within the IEA Greenhouse Gas R&D Program website will be updated to communicate the vision and activities of the Network. Specifically, the Website will include the Technology Roadmap, as well as an updated and extended descriptions of the Network and activities, as well as posting of the Minutes of this as well as those of prior meetings, likely in redacted format. Details will be discussed at the next meeting in Paris. Reserving access of specific information to Members would allow posting of a more extensive data sets and information. Plans for upgrading of the Website will be discussed prior to the September 29th meeting, and be implemented after that meeting based on Member feedback. Member Organizations, Technical Advisers and others will be asked to provide information for inclusion on the website and/or links to other relevant sites. This work will be carried out by the Network Manager and IEA GHG R&D Programme staff, specifically Angela Manancourt, who now also supports the Network management.

c. Project Development. The main function of the Network is to help Member Organizations to initiate new and carry out ongoing R&D projects in a cooperative and collaborative manner. Current and proposed projects are listed in Sections C1 and C2, covering much of the R&D needs outlined in the Technology Roadmap. Additional projects and R&D activities are still required in several areas. Such projects may be developed by new and existing Members with assistance of the Network team.

d. Membership Relations and Recruitment. A major objective of the Network for the present year is to expand membership in the Network. Recruitment efforts will be initiated in the run-up to the next Technical Meeting in Paris, September 29th. Participation by small businesses, and not-for-profits will be addressed during that meeting. However, the greater effort will be to support the R&D activities of existing Members and in member relations. For examples, greater involvement by the U.S. Dept. of Energy in Network activities will be sought, new projects developed and coordination and collaborations among R&D projects sought and strengthened.

F. CONCLUDING COMMENTS

The goal of the Network is to assist Member Organizations to carry out R&D projects in microalgae greenhouse gas abatement that meet their needs and goals and to provide opportunities for project coordination, collaboration and integration. This is, of course, an organic process which will require some time to come to full fruition and will need to fit the individual interests and requirements of the Member Organizations. The first year of the Network has seen considerable progress toward these goals, highlighted at the Berkeley Meeting. That meeting promoted interactions among Members and other participants and provided an overview of what has already been achieved and what is possible and needs to be accomplished.

The six already ongoing projects by the Member Organizations (not counting ExxonMobil) are sufficient by themselves (see C.1, page 17) to justify the existence and activities of the Network. The funding of these projects is significant, at over U.S.$ 2 million per year. Three of those projects (Enitecnologie and the two U.S. DOE projects) are already part of a well integrated and coordinated effort, through collaborative activities among the researchers (e.g. strain and data exchanges). The other three projects, currently operated independently, are expected to also cooperate and collaborate to various degrees in the overall effort during the coming year.

To these ongoing projects by the Member Organizations can be added the half dozen R&D projects, proposed or related to the Network, developed by the Network Manager and Technical Advisers (see C.2, page 18, for summaries). Most advanced is the Salton Sea "CEP Project", already ongoing for a year, although not yet a project sponsored by a Member Organization. One project proposed by a Network participant (Joe Weissman, SeaAg, Inc., SBRI Phase I and II, SeaAg, Inc.) was been funded by the U.S. DOE and thus has became a Network project. Other projects are still in the developmental phase. These projects have for their common goal to move this technology beyond the current stage by achieving much higher productivities in open ponds for higher value (than just fuels) applications, as outlined in the Technology Roadmap.

The Technology Roadmap is the single most important work product deriving from the Network over the past year. It provides an R&D strategy for development of practical microalgae GHG abatement processes. It focuses on one type of technology – open, raceway, paddle wheel mixed ponds supplied with power plant flue gas CO_2. This general technology includes several very different applications, from municipal wastewater treatment to agricultural nutrient recycling and fertilizer production, from aquaculture systems to production of specialty animal feeds and other large volume commodities. The main GG abatement mechanisms are the renewable biofuels produced and the reduced energy inputs compared to conventional technologies.

Overall, the Network has made substantial progress over its first year, though much remains to be accomplished. However, in actuality, it is not the Network that makes progress but the individual R&D projects being carried out or sponsored by the Member Organizations that advance this technology. And such advances, for example in the key area of productivity increases, will take a several years to come to full fruition. Thus, for the present, accomplishment can be gauged by the number and quality (funding, focus, coordination, etc.) of existing projects supported and new ones initiated by the Network Members. The objective of the Network is to achieve the goals of the Technology Roadmap through increased in R&D in this field. This will be accomplished through technical assistance to existing Members and recruitment of new ones.

APPENDIX A. FIELD TRIPS AND BANQUET

1. Visit to St. Helena Wastewater Treatment Plant

This bus field trip took place on Sunday, April 27th, and visited the Napa Valley, to inspect the City of St. Helene, municipal wastewater treatment plant using the AIWPS® technology (See presentation by Dr. Bailey Green). Dr. Green gave an explanation of the process and system at St. Helena. The system has undergone some important changes over the years, in particular with modifications of the influent lagoon, and change from discharge to the adjoining Napa River to irrigation of adjoining pastures and orchards. These changes are still ongoing, with paddle wheels being planned for replacing the pumps currently providing circulation and a DAF (dissolved air floatation) unit for algal harvesting being currently tested.

After the visit to the St. Helena wastewater ponds, the tour visited the Domain Chandon winery, where its was hosted by Mr. Thomas Tiburzi, a former student and employee of John Benemann and Joe Weissman, and now Associate Wine Maker at this prestigious winery. Mr. Tuburzi provided a detailed explanation of how wine and champagne is made, from the grape in the field to the bottle on the shelf. The great sophistication of the process suggests that microbiological processes require considerable research and practical development, but can be accomplished.

2. Banquet – Appreciation for Prof. William J. Oswald (28th April)

The Banquet was held at the Men's Faculty Club of the University of California Berkeley, with Prof. William Oswald as guest of honor. Prof. Oswald was the mentor of many of the scientists and engineers working with microalgae today, including, among the participants at this meeting, Drs. Benemann, Green, Hallenbeck, and Weissman. His pioneering work in wastewater treatment, space exploration, animal feeds, energy production and many other applications of microalgae was an inspiration to all researchers in this field. Dr. Oswald encouraged the participants to keep up with their work and predicted that microalgae technologies would be able to solve many of our environmental and resource problems in the 21st Century.

3. Visit to Sunnyvale Wastewater Treatment Plant (April 29th)

The City of Sunnyvale California, in the heart of Silicon Valley and on the shore of the San Francisco Bay, operates a 440 acre (180 hectares) algal pond system for municipal wastewater treatment. The incoming wastewater is sedimented (primary treatment) and the overflow sent by a pipe to a distribution channel that introduces the primary treated waste to two oxidation ponds (appx. 320 and 120 acres, each). Large circulation pumps set up some flow in the ponds. The pond waters are then pumped back to the treatment plant where a DAF (dissolve air floatation) unit is used to flocculate (with cationic polymers) and harvest (floatation) the algal biomass. Effluent polishing is accomplished with nitrification, mixed media filtration and chlorination. The harvested algae, along with primary sludge are used to produce methane gas in four anaerobic digesters. This biogas is combined with landfill gas and natural gas (typically in a ratio of 60 -25- 15) and used to generate electricity in two 800 kW(e) caterpillar engines. Sunnyvale is a potential site for using the flue gas from these generators for an algal production process (see Section C.2.e).

APPENDIX B. SELECTED REFERENCES

This Appendix provides a preliminary list of references of interest in the context of this Meeting. A more extensive list will be posted on the Network website. The following two publications are available on request from Dr. Heino Beckert, U.S. DOE, NETL (Heino.Beckert@netl.doe.gov).

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B. Benemann, J.R., and W.J. Oswald, *Systems and Economic Analysis of Microalgae Ponds for Conversion of CO2 to Biomass*, Final Report to the Pittsburgh Energy Technology Center under Grant No. DE-FG22-93PC93204, pp.260. March21, 1996

1. Presentation by Paola Pedroni (Network, Comparison of ponds and photobioreactors)

A. Pedroni, P., J. Davison, H. Beckert and P. Bergman, and J. Benemann. "A Proposal for an International Network on Biofixation of CO2 and Greenhouse Gas Abatement with Microalgae". Proceedings, National Technology Energy Laboratory/Dept. of Energy, 1st National Conf. on Carbon Sequestration. May 2001 (2001).

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C. Benemann, J.R., Pedroni, P., J. Davison, H. Beckert and P. Bergman, "Technology Roadmap for Biofixation of CO_2 and Greenhouse Gas Abatement with Microalgae", Presented at the 2nd Annual C Sequestration Conf., U.S. Dept. of Energy, NETL, Alexandria, Virginia, May 2003

2. Presentation by David Barr (RuBisCo enzyme)

B. "Maxygen Announces Bioprocess Development Collaboration with Rio Tinto", 1/20/2000 Codexis Announces Extension of Collaboration with Rio Tinto 2/27/2003 (www.Codexis.com).

3. Presentation by Roger Prince (Botryococcus *Braunii*):

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B. Hu, Q., D. Faiman and A. Richmond. Optimal orientation of enclosed reactors for growing photoautotrophic microorganisms outdoors. J. Ferment. Biotech. 85:230-236 (1998).

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d. Richmond, A. and Q. Hu. Principles for utilization of light for mass production of photoautotrophic microorganisms. Appl. Biochem. Biotechnol. 63-65:649-658 (1997).

5. Presentation by Bailey Green

a. Green F.B., Lundquist T.J. and Oswald W.J. 1995. Energetics of advanced integrated wastewater pond systems. Water Sci. Technol. 31(12): 9-20.

B. Green F.B.(1998). The Energetics of Advanced Integrated Wastewater Pond Systems. Ph.D. Dissertation, Energy and Resources Group, Univ. Calif., Berkeley.

6. Presentation by David Brune (Salton Sea Project)
A. Brune, D. E., J. A. Collier, and T. E. Schwedler, "Partitioned Aquaculture System," United States Patent No. 6,192,833, February 2001.
B. Brune, D.E., Hong Wei-Yen, J. Van Olst, M.J. Massingill, J.M. Carlberg and J.R. Benemann, "Integrated Production of Biofuel, Biofertilizer, and High Value Aquatic Biomass in a Controlled Eutrophication Process," Proceeding International Conference: Bioenergy 2002
C. Benemann, J. R., J. C. Van Olst, M. J. Massingill, J.C. Weissman, and D.E. Brune, "The Controlled Eutrophication Process: Using Microalgae for CO_2 Utilization and Agricultural Fertilizer Recycling," Proceeding GHGT-6, Kyoto Japan, October, 2002.
D. Brune, D.E., G. Schwartz, J. R. Benemann, M. J. Massingill, J. C. Van Olst, and J. A. Carlberg. "The Controlled Eutrophication Process: Microalgae Biofixation of CO 2 for Biofuels Production and Fertilizer Recycling at the Salton Sea, California" Presented at the 2nd Annual C Sequestration Conf., U.S. Dept. of Energy, NETL, Alexandria, Virgina, May 2003

7. Presentation by Michael Huesemann (Growth Rates and Productivity).
a. Huesemann, M., R. Bartha and T.S. Hauseman and J. Benemannn, "An Innovative Approach for Screening Marine Microalgae for Maximum Flue Gas CO2 Biofixation Potential". Presented at the 2nd Annual C Sequestration Conf., U.S. DEO -NETL, Alexandria, Virginia, May 2003

8. Presentation by Joseph Weissman (Productivity in open ponds and photobioreactors)
a. Weissman, J.C., and J.R. Benemann "Comparison of Marine Microalgae Culture Systems for Fuels Production and Carbon Sequestration", Presented at the 2nd Annual C Sequestration Conference, U.S. Dept. of Energy, NETL, Alexandria, Virginia, May 2003
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10. Presentation by Patrick Hallenbeck. (Biohydrogen).
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