



## PURPOSE

Bio-inspired Hydrogen and BioHydrogen (microbial) production processes have been active fields of basic and applied research for many years, with significant R&D programs currently carried out around the world. Task 21 is carrying out collaborative research activities in areas, which include H<sub>2</sub> production using in vitro, biomimetic, and artificial photosynthetic systems; photosynthetic microbes; dark bacterial fermentations; biological/enzymatic fuel cells; and integrated combinations of these technologies. The overall objective is not only to sufficiently advance basic and applied science in these areas of research over a five-year period, but also to evaluate these technologies from the perspective of economics and sociology. A five-year period is considered to be sufficient time to initiate a significant directed research program, set metrics for evaluation of the developmental status and promise of this field of research and technology, and achieve some major advances.

## FRAMEWORK SUMMARY

The Task covers the following 5 subtasks: A. Bio-inspired systems (i.e., identify and develop promising applications of biomimetic, in vitro, and artificial photosynthetic H<sub>2</sub>-producing processes—Marc Rousset, France, Subtask Leader); B. Dark biohydrogen fermentation systems (i.e., increase achievable H<sub>2</sub> production from substrates above the currently achievable yields—Patrick Hallenbeck, Canada, Subtask Leader); C. Basic studies for light-driven biohydrogen production (demonstrate potential practical processes for conversion of water or organic substrates to H<sub>2</sub> with solar energy input—Peter Lindblad, Sweden, Subtask Leader); D. Biological electrochemical systems (identify and develop promising applications of microbial/enzymatic electrochemical cells for H<sub>2</sub>-production processes, Alan Guwy, UK, Subtask Leader); and E. Overall analysis (determine how to introduce bio-inspired hydrogen and biohydrogen processes as new technologies in support of the coming H<sub>2</sub> society; analyze technologies from the economic, technological, and societal point of view—Jun Miyake, Japan, Subtask Leader).

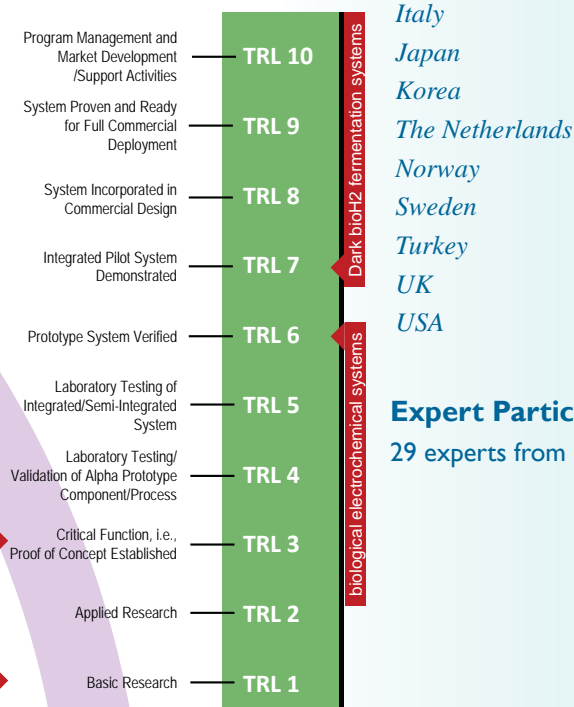
## STATUS OF THE TECHNOLOGY

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**Subtasks A—D** have major Basic Research, Applied Research, and Critical Function elements; Subtasks B and D in addition have Laboratory Testing and Prototype System Verification elements; Subtask B has an Integrated Pilot System Demonstration element; and Subtask E has a Program Management and Market Development element.

### TECHNOLOGY READINESS LEVEL

Dark biohydrogen fermentation systems are at the Technology Readiness Level (TRL) 7 (Integrated Pilot System Demonstration) level and biological electrochemical systems are at the TRL 6 (Prototype System Verification) level. All other projects in Subtasks A-D are at the TRL 1–3 (Basic Research,



## TASK 21

### BIO-INSPIRED HYDROGEN AND BIOHYDROGEN

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### VITAL STATISTICS

#### Term

Phase 1: 2010-2013

Phase 2: 2013-2015

#### Members

Australia

Canada

Finland

France

Germany

Italy

Japan

Korea

The Netherlands

Norway

Sweden

Turkey

UK

USA

#### Expert Participants

29 experts from 14 countries





**2011 Meetings**  
February 28-March 1, 2011

**Singapore**

**September 6-7, 2011**

**Istanbul, Turkey**

Applied Research, and Critical Function, respectively) levels with the exception of Subtask E, which is a TRL 10 (Market Analysis) task.

## MEMBERS

### TASK MEMBER AND EXPERT TABLE (ATTACHED HERE AND PREVIOUSLY PROVIDED)

COUNTRY	NAME	COMPANY/UNIVERSITY (CATEGORY)
Australia	Ben Hankemar	University of Queensland (Univ)
Canada	Hallenbeck, Patrick Subtask Ldr	Dept. of Microbiology and Immunology, University of Montreal (Univ)
France	Rousset, Marc Subtask Ldr	CNRS Marseille (Gov)
France	Cournac, Laurent	CEA Cadarache (Gov)
Finland	Puhakka, Jaakko	Department of Chemistry and Bioengineering, Tampere University of Technology (Univ)
Finland	Aro, Eva-Mari	University of Turku (Univ)
Germany	Schulz, Rüdiger	Botanisches Institut und Botanischer Garten, Christian-Albrechts-Universität (Univ)
Italy	Torzillo, Giuseppe	CNR - Istituto per lo Studio degli Ecosistemi (Gov)
Italy	De Philippis, Roberto	Dept. of Agricultural Biotechnology, University of Florence (Univ)
Japan	Miyake, Jun Subtask Ldr	Dept. of Mechanical Science and Bioengineering, University of Osaka (Univ)
Japan	Tomiyama, Masamitsu	National Institute of Agrobiological Sciences
Japan	Wakayama, Tatsuki	INPEX Corp. (Ind)
Korea	Kim, Mi-Sun	Bioenergy Research Center, Korea Institute of Energy Research (Res. Inst.)
Korea	Kim, Dong Hoon	Bioenergy Research Center, Korea Institute of Energy Research (Res. Inst.)
The Netherlands	Stams, Fons	Wageningen UR & Food Biobased Research (Univ)
The Netherlands	Mars, Astrid	Wageningen UR & Food Biobased Research (Univ)
The Netherlands	Kergen, Serge	Wageningen UR & Food Biobased Research (Univ)
Norway	Skjånes, Kari	Bioforsk (Ind)
Norway	Birkeland, Nils-Kåre	University of Bergen (Univ)
Sweden	Lindblad, Peter	Microbial Chemistry, Dept. Photochemistry and Molecular Science, The Ångström Laboratories, Uppsala University (Univ)
Sweden	Sellstedt, Anita	Dept. of Plant Physiology, Umeå University (Univ)
Turkey	Eroglu, Inci	Dept. of Chemical Engineering, Middle East Technical University (Univ)
Turkey	Yucel, Meral	Dept. of Biological Sciences, Molecular Biology & Biotechnology R&D Center, Middle East Technical University (Univ)
Turkey	Öztürk, Yavuz	Genetic Engineering and Biotechnology Institute (Gov)
United Kingdom	Guwy, Alan J. Subtask Ldr	University of Glamorgan (Univ)
United States	Seibert, Michael	Energy Sciences Directorate, NREL (Gov)
United States	Maria Ghirardi	Photobiology Group, NREL (Gov)

## GROWTH/CHANGES IN MEMBER AND/OR EXPERT PARTICIPANT COMPOSITION

Since the last Annual Report, Task 21 has added one new country (Australia), added two new Experts, and lost one Expert.

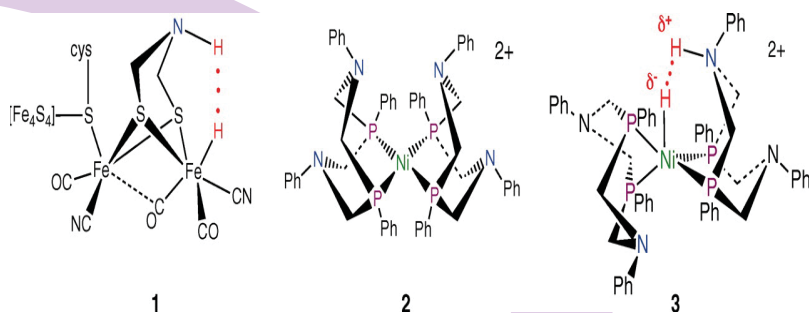
## ACTIVITIES AND RESULTS IN 2011

### PROGRESS AND ACCOMPLISHMENTS

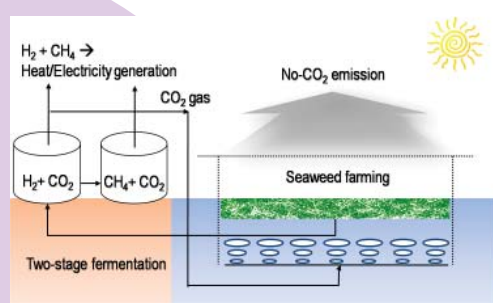
#### Subtask A. Bio-inspired H<sub>2</sub>-producing Systems

Three groups from the USA and one group from Sweden reported significant advances in the synthesis of water-oxidation catalysts using earth abundant metals (Co, W, P, and Mn) for use in artificial photosynthetic systems to produce reductant for the generation of H<sub>2</sub> from water. Mechanisms to explain the water-oxidation process were also advanced. Other groups from the USA reported complexes that can catalytically generate gaseous H<sub>2</sub> either from water at neutral pH or from sea water and that might reveal the potential roles of the metals found in the natural photosynthetic Mn<sub>4</sub>Ca water-oxidizing cluster. At least three other USA groups are also working on similar catalysts. Furthermore, groups from France and the USA reported H<sub>2</sub>-production catalysts that mimic the activities of [FeFe]- and [NiFe]-hydrogenases (hydrogenases—enzymes that microbes use to release H<sub>2</sub> gas). The idea here is to develop replacements for natural enzymes for use in constructing biomimetic or artificial photosynthetic systems that produce H<sub>2</sub>, or in the case of hydrogenase mimics, replace the need platinum in fuel cells.

A key milestone (demonstrate the synthesis of functioning water-oxidation and H<sub>2</sub>-production catalysts that do not contain noble metals) was reached by the DuBois group (PNNL, USA), who reported that a synthetic nickel complex, [Ni(PPh<sub>2</sub>NPh)<sub>2</sub>](BF<sub>4</sub>)<sub>2</sub> (see Figure) catalyzes the production of H<sub>2</sub> with record turnover frequencies of 33,000 s<sup>-1</sup> in dry acetonitrile and 106,000 s<sup>-1</sup> in 1.2 M water at a low over potential.



#### Subtask B. Dark Biohydrogen Fermentation Systems



This subtask is one of the most active, with five countries having reported contributions in the last year. Researchers from (i) Canada reported that application of sonic energy directly inside a fermentation bioreactor (SBHR) almost doubles the H<sub>2</sub>-production rates and yields, and that zeolite adsorbents can be used to purify



fermentation gas streams (separate CO<sub>2</sub> from H<sub>2</sub>); (ii) Finland's H<sub>2</sub>-production studies using dark fermentation focused on the effects of chemical and biological hydrolysis of cellulose for subsequent fermentative H<sub>2</sub> production. Microbial cultures producing H<sub>2</sub> from these substrates and operating at elevated temperatures (59–78°C) were successfully enriched and characterized from different origins, such as hot springs, an underground mine, compost, and cow rumen. Effects of process parameters, metabolic pathways, and microbial communities were also reported; (iii) Korea in collaboration with Norway reported the first use of marine algae as a feedstock for dark, fermentative H<sub>2</sub> production, and Korea, exploring the use of sonication, found that algae cells were significantly damaged during sonication, that carbohydrates diffused out of the microalgal interiors, increasing bioaccessibility and bioavailability of the biomass, and that the cumulative bioenergy (ethanol/H<sub>2</sub>) production after long-term sonication was almost 7 times higher than that after short-term treatment or the control; and (iv) Sweden reported a hydrogenase, found in *Frankia*, with high O<sub>2</sub> tolerance that shows potential for expression in algal or cyanobacterial systems for use in improving H<sub>2</sub> photoproduction.

In the completion of a key milestone (scale up a H<sub>2</sub>-producing fermentation technology with industrial support), Japan reported that the Sapporo Brewery in collaboration with Petrogas and the Ergostech Company in Brazil is constructing a pilot plant to produce Biohydrogen from beer waste water in a plant located in Campinas, Brazil.

#### **Subtask C. Basic Studies of Light-driven BioH<sub>2</sub> Production**

Examples of many technology advances in this area are listed: (i) Germany reported antenna-depleted cyanobacterial mutants generated to increase the effective content of photosystem II reaction centers in the organism. The antenna-mutants also exhibited higher cell density. As reported below with the green alga, *C. reinhardtii*, the goal of this approach is to increase the amount of H<sub>2</sub> that can be produced per unit photobioreactor surface area; (ii) Finland screened the UHCC (Univ. of Helsinki Cyanobacterial Culture) collection for strains that exhibited enhanced H<sub>2</sub> photo production. The 10 best strains were further examined for optimal culture conditions to enhance their capacity for H<sub>2</sub> production. Furthermore, *Chlorella* species were immobilized within a thin alginate film in order to achieve long-term H<sub>2</sub> production as has been done with bacteria and algae below; (iii) Italy demonstrated the feasibility of producing H<sub>2</sub> outdoors, using the photosynthetic bacterium, *R. palustris*, and that the culture performance can be improved with a better photobioreactor design (currently hampered by an overly long mixing time). Sustained H<sub>2</sub> photoproduction was also observed in *Rhodospseudomonas palustris* GCA009 NifA1976 (obtained from Harwood, USA), which is impaired in ammonia-regulation of its nitrogenase. The mutant showed undiminished H<sub>2</sub> production even in the presence of 10 mM ammonia, which will increase the range of substrate material that this type of organism can process; (iv) Norway constructed a new type of bioreactor system for algal cultivation and subsequent H<sub>2</sub> production. The bioreactor design (in collaboration with India) is based on a flat plate reactor, which can be used in two different ways. For algae cultivation, the culture compartment is positioned vertically with an air bubbling tube for mixing and CO<sub>2</sub> addition. For the following H<sub>2</sub>-production step, the culture compartment of the reactor is positioned horizontally with a rocking motion facilitated by an engine/gearbox where the rocking speed and angles can be varied; (v) Turkey examined photofermentative H<sub>2</sub> production with agar-immobilized *R. capsulatus* DSM1710 and *R. capsulatus* YO3 (hup-) in 1L panel photobioreactors. Compared to suspension

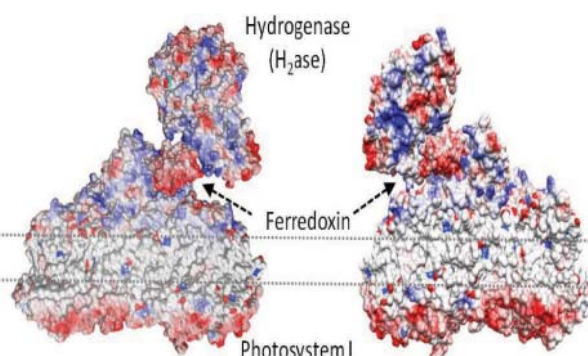


cultures, immobilization resulted in higher yields and productivities over 60 days under indoor conditions with continuous illumination. Outdoor experiments with liquid cultures of these strains were carried out during winter months to test low temperature and light, and summer months to test high temperature and light on biomass yield, H<sub>2</sub> productivity, and H<sub>2</sub> yields. A linear relationship was observed between the daily yield factor (mol H<sub>2</sub>/gdcw) and the daily total global solar radiation. Turkey also demonstrated continuous photofermentative H<sub>2</sub> production on real, dark fermenter effluents in stable scale-up operations for up to 5 months using 4L panel photobioreactors. A pilot scale (100L) tubular photobioreactor with internal cooling coils was also operated under outdoor conditions using dark fermenter effluents of thick juice and molasses; and (vi) The USA reported a new H<sub>2</sub>-producing marine cyanobacterium that can produce large amounts of H<sub>2</sub> (up to 900 ml/l in 2.5 days) photomixotrophically under aerobic conditions in the presence of 50 mM glycerol, the transformation of a clostridial [FeFe]-hydrogenase in a cyanobacterium, provided definitive proof that algae with a truncated antenna produce more H<sub>2</sub> per culture surface area at high light intensity than do WT algae, and showed that a fused photosystem I-ferredoxin-hydrogenase protein complex (see figure) in vitro preferred H<sub>2</sub> production vs NADP<sup>+</sup> reduction (CO<sub>2</sub> fixation). This last work demonstrated that it is possible to engineer a bio-hybrid system that can overcome the natural tendency of photosynthesis to fix carbon rather than produce H<sub>2</sub>.

#### Subtask D. Biological Electrochemical Systems

In a microbial electrolysis cell (MEC), electricity is used for the reduction of protons to H<sub>2</sub> gas at the cathode, using electrons derived from the degradation of organic compounds at the anode. The Netherlands reported that to reduce cost the metabolic properties of *G. sulfurreducens* for acetate oxidation at the anode and H<sub>2</sub> production at the cathode were combined in one-compartment, membraneless MECs operated at applied voltages of 0.8 and 0.65 V. H<sub>2</sub> was produced in this configuration.

Fulfilling a key milestone (demonstrate a pilot-scale MEC device), researchers from Penn State with engineering services supplied by Brown and Caldwell announced the first small industrial-scale demonstration of an MEC device (see figure) for BioH<sub>2</sub> production at the Napa Wine Company, in Oakville, CA



Model of the interaction between the algal hydrogenase, ferredoxin, and PSI complexes; the model guided the construction of a fused Ferredoxin/Hydrogenase protein. Red amino acids = acidic (-); blue = basic (+); and white = non-polar.





### Subtask E. Overall Analysis

Two major analysis efforts have been completed. The first, a feasibility study, released by Hiroshima University in Japan, outlining an integrated system that would use waste glycerol (from biodiesel production) to produce a suite of fuels, including additional biodiesel, H<sub>2</sub>, and ethanol. The second was a new Science article, published by a group of distinguished researchers in the USA, which compared photosynthetic and photovoltaic solar conversion efficiencies. The analysis considered opportunities in which the frontiers of synthetic biology might be used to enhance natural photosynthesis for improving solar energy conversion efficiency.

Japan reported the completion of a milestone (develop cost information for the industrial scale-up of a 2-stage Biohydrogen/ Biomethane plant) related to the Sapporo Brewery/ Petrogas/ Ergostech Company pilot plant collaboration in Brazil. Sapporo carried out a feasibility study estimating the projected capital cost of a small scale (\$36 million) and large-scale (\$1.2 billion; ~40JPY/Nm<sup>3</sup>-H<sub>2</sub>) BioH<sub>2</sub> facility. A small facility is under construction at the Ergostech Company facility near Sao Paulo, Brazil.

## OUTREACH AND COMMUNICATION

### Summary of Strategy and Activities

Task 21 has been active in promoting research collaboration between member countries. Currently there are 17 formal collaborations between member countries and internal collaborations among four countries, all catalyzed by the association with Task 21.

The most transparent means of outreach and communication is through the peer review literature and presentations at international meetings. Task meetings, whenever possible, are held as satellite meetings of large international conferences (the last two task meetings were held in conjunction with the Keystone Symposium on Biofuels in Singapore and SET 2011, the 10th International Conference on Sustainable Energy Technologies in Istanbul).

Task 21 Communication and Outreach Table 2011

PUBLICATION / PRESENTATION NAME	PUBS	PRES	OTHER
Task 21 "Bio-inspired Hydrogen and Biohydrogen"	204		
Ph.D. dissertations	15		
M.S. theses	2		
Patents	11		
Task 21 "Bio-inspired Hydrogen and Biohydrogen"			1
Lectures at scientific meetings and universities		28	
Task 21 "Bio-inspired Hydrogen and Biohydrogen"			1
Task 21 "Bio-inspired Hydrogen and Biohydrogen"			1
Task 21 "Bio-inspired Hydrogen and Biohydrogen"			1
<b>TOTAL</b>	<b>232</b>	<b>28</b>	<b>4</b>

Task 21 Experts reported 204 peer-review publications, including 18 publications in high impact journals (Nature, Science, PNAS, and JACS) and 54 publications in the International Journal of Hydrogen Research (the world's premier H<sub>2</sub> research journal).





### Task websites

Task 21 maintains a private website for the benefit of all task experts. The site is a repository for country report presentations given at semi-annual task meetings, country and task semi-annual reports, task meeting minutes, lists of task publications, and other materials that task Experts might find useful.

## REFERENCES

### SELECTED KEY PUBLICATIONS

Please see Task 21 Bibliography on IEA HIA website at <http://ieahia.org/pages/static/task21.htm>.