A New IEA/HIA Task: Bio-inspired and Biological Hydrogen -- March 22, 2010 Final

Work Program

For Task 21 Extension Official Proposal at the EXCO Meeting in May 2010

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1. Introduction

Bio-inspired Hydrogen (*in vitro,* biomimetic, and artificial photosynthetic H_2 production processes) and BioHydrogen (the production of H_2 by microorganisms) have been active fields of basic and applied research for many years, with significant applied R&D programs currently supported by the Governments of Japan, Europe and the U.S., as well as related basic research also carried out in these and other countries. First, the realization of practical processes for H_2 production from water using solar energy would result in a major, novel source of sustainable and renewable energy, without greenhouse gas emissions or environmental pollution. This important and challenging area was studied during previous Task periods. However, new ideas have become available and development of practical processes will require additional significant scientific and technological advances, and relatively long-term basic and applied R&D. Our previous efforts to develop photobiological H_2 production from water and dark H_2 production (fermentative processes using waste biomass) have resulted in substantial scientific progress and should be continued. Furthermore, new emphasis should be placed on bio-inspired and artificial photosynthesis approaches for the production of H_2 from water using biological processes as a template. Hydrogen production by *in vitro* systems will also include nano-technology and molecular engineering technology. In addition, integrated biological (including combinations of photobiological, fermentative processes, biological/enzymatic fuel cells for cost containment) opportunities will also be emphasized in an extended Task. Finally, the economic analysis of proposed technologies and social acceptance of such technologies will also be emphasized as a new subtask. As the cost of energy rises and the environmental consequences of traditional forms of energy become more apparent, new third generation energy technologies, such as those to be investigated in this Task extension, must be developed to supplement and ultimately replace current technologies. We must evaluate the feasibility of these technologies, taking every factor into consideration. The extended Task will cover the above areas of R&D, technological/economic evaluations, and societal acceptance, which are of mutual interest to the countries and researchers participating in the IEA Hydrogen Implementing Agreement. This Task will provide a basis for establishing real collaborative research projects and an overall coordinated program.

2. Objective

The Task will carry out collaborative research activities on the production of H_2 using *in vitro,* biomimetic, and artificial photosynthetic systems; photosynthetic microbes; bacterial dark fermentation; biological/enzymatic fuel cells; and integrated combinations of these technologies. The overall objective is not only to sufficiently advance the basic and applied science in this area of research over the next five years but also to evaluate these technologies from the aspects of economics and sociology. A five-year period is considered to be a sufficient time period to initiate a significant directed research program and achieve some major advances and metrics for evaluation of the developmental status and promise of this field of research and technology.

3. Task Approach

A five-year collaborative R&D program is proposed. The Task is scheduled for a three-year period, with an option for a 2-year extension. The Task concerns the development of basic and applied Bio-inspired H_2 and BioHydrogen sciences and the total integration of components of these technologies into cost competitive system. H_2 production by microalgae, cyanobacteria, and bio-inspired systems from water as well as H_2 dark fermentation from biomass and its combination with photofermentation and/or biological/enzymatic fuel cells will be also be studied from a techno-economic evaluation and social acceptance perspective. The following R&D areas will be investigated in a collaborative R&D effort.

4. Subtasks

The Task consists of 5 subtasks to be based on the technological field. Subtasks to be included under this Task are as follows:

Subtask A. **Bio-inspired Systems**.
Goal: To identify and develop promising applications of *in vitro*, biomimetic, and artificial photosynthetic H_2-production processes.

Hydrogen production by *in vitro*, biomimetic, and artificial photosynthetic systems will involve nano-technology and molecular engineering technologies. In *in vitro* systems for H_2 production, parts of the photosynthetic apparatus from cyanobacteria, algae or photosynthetic bacteria along with hydrogenase enzymes from various kinds of bacteria are separated and artificially re-constituted to maximize light-conversion efficiency to H_2. Biomimetic systems will combine biological and non-biological components to maximize light-conversion efficiency to H_2. Finally, artificial photosynthetic processes will totally replace biological components with the goal of developing man-made, self-assembling systems to maximize light-conversion efficiency to H_2 using long-lived, non-hazardous, low cost materials. These technologies will address problematic limitations in the efficiency and stability of natural photosynthetic systems. Catalysts that are developed under this Subtask would be as efficient as biological catalysts, but not as expensive as those based on metals such as Pt and Pd. Biomimetic systems will also be aimed at mimicking the hydrogenase active site. One limitation in the large scale development of fuel cells (Subtask D) is that the Pt and Pd, which currently constitute the electrodes, are a limited resource. In contrast the active site of hydrogenases is composed of two of the most abundant metals on earth (Fe and Ni). The ability to create new catalysts based on these metals, inspired by hydrogenases, would remove constraints on the capability of producing fuel cells at significantly decreased cost.

Subtask A will focus on:
i. Enzyme systems for H_2 production.
ii. Bio-inspired systems for H_2 production.
iii. Artificial photosynthesis for H_2 production.

Activity Leader: Peter Lindblad (Sweden)

<u>Subtask B</u>. Dark BioHydrogen Fermentation Systems.
Goal: To increase achievable H_2 production from substrates above currently achievable yields (e.g., to ≥ 4 moles H_2/mole of glucose equivalent).

The production of H_2 by dark fermentation using biomass, waste biomass, or organic wastes (sewage) as substrate for fermentative bacteria is an important nearer-term area for BioHydrogen R&D. Biomass and organic wastes are renewable because they are products of photosynthesis. Because of the basic knowledge available about the biology of dark fermentations, H_2 from fermentation at the current time is probably the BioHydrogen technology closest to practical application. However, there are technological challenges including the relatively low yields of H_2 per unit of waste material consumed, the amount of waste acid production, the limited rates of H_2 production, and the cost and recalcitrance of potential biomass feedstocks. To address these, two-stage, integrated systems incorporating (a) a dark fermentative process and a biological electrochemical (fuel cell) system (coordinated with Subtask D) and (b) dark and photofermentation systems (coordinated with Subtask C) will be examined. The goal of these integrated systems would be to increase the yield of H_2 close to 12 moles of H_2 per mole of glucose equivalent. System costs could be decreased if the processes could be linked to improvements in the efficiency of cellulose deconstruction.

Subtask B will focus on:
i. Metabolism, genetics and thermodynamics of H_2-producing bacteria to identify critical genes, pathways and regulatory components for high yield H_2 production.
ii. Genetic and physiological manipulation to maximize H_2 production—identification of bacteria, the diversity of hydrogenases in different organism, and conditions that allow for high H_2-production rates.
iii. Fermentative H_2 production from low cost, biomass/organic substrates in the dark under conditions that produce large amounts of H_2 and minimal acid waste (this latter topic can be addressed in Subtask D).

Activity Leader: Patrick Hallenbeck (Canada)

<u>Subtask C</u>. Basic Studies for Light-driven BioHydrogen Production.
Goal: To demonstrate potential practical processes for conversion of water or organic substrates to H_2 with solar energy input.

Although H_2 evolution mediated by hydrogenase(s) was discovered in green algae almost seventy years ago and subjected to extensive investigations over some of the ensuing decades, there are still many important fundamental and applied issues that must be addressed before this technology can be considered for practical applications. Hydrogen production from organic substrates using anoxygenic photosynthetic bacteria has also been extensively studied to improve efficiencies. The objectives of this Subtask will require fundamental understanding of the genetics, biochemistry, physiology, and systems biology of H_2-producing enzymes and organisms, including the metabolism and factors affecting growth of photosynthetic microbes. Furthermore, the efficiency of photosynthesis (the conversion of light energy to biochemical energy) ultimately limits the efficiency of H_2 production. Understanding photosynthetic mechanisms in relationship to H_2 production and improvements to photosynthetic efficiency are ambitious targets to address. Advancing the potential of oxygenic, photosynthetic organisms into optimal H_2-production processes will require the design of dedicated cultivation systems (photobioreactors) and elaboration of suitable cultivation protocols and system-control procedures (software). Realizing these objectives will require the application of modern and advanced tools of molecular biotechnology, systems biology, microbial physiology, biotechnology, and bioprocess engineering, techniques already available at leading research laboratories in the participating countries. An ultimate potential objective might be to increase BioHydrogen production as close as possible to about 600,000 m^3 ha^{-1} y^{-1}, which represents solar light conversion efficiency to H_2 of about 10% in sunny areas. Furthermore, the major problem with long-term continuous photofermentative H_2 production is stable operation, and bio-sensing systems in photosynthetic bacteria (as well as oxygenic phototrophs) may have a role in H_2 production. Therefore, these systems must be investigated for detailed understanding of the regulation of H_2 production and its relationship to other metabolic pathways. It is necessary to explore signal transduction pathways in response to critical physiological factors such as pH, light intensity, C/N ratio, and temperature. The goal is to increase the stability of the long term, continuous photobioreactor applications and development of novel process control systems. Finally, coordinated applications with Subtask B will include adding photofermentation inline after a dark, fermentation process to increase the total molar stoichiometry of H_2 production per glucose equivalent close to the theoretical limit of 12.

Subtask C will focus on:
i. Genetics, metabolism, and systems biology of H_2 production by photosynthetic microbes.
ii. Fundamental studies of hydrogenases and their susceptibility to O_2.
iii. Selection of algal strains with high H_2 output.
iv. Physiology and biotechnology of photosynthetic microbes to maximize H_2 production from water or organic wastes.
v. Photosynthesis under stress conditions—overcoming limiting factors.
vi. Development of photobioreactor designs for H_2 production outdoors.
vii. Examination of bio-sensing in phototrophic organisms.
viii. Immobilized systems to increase productivity and yield of photofermentation.

Activity Leader: Marc Rousset (France)

Subtask D. **Biological Electrochemical Systems**.

Goal: To identify and develop promising applications of microbial/enzymatic electrochemical cells for H_2-production processes.

Hydrogen production by construction and utilization of microbial/enzymatic electrochemical systems (fuel cells) will involve attaching microbes or enzymes directly to electrodes in biological fuel cell configurations to produce H_2 from waste organic materials or waste biomass. Coordinated applications with Subtask B will include adding a biological fuel cell inline after a dark or photo fermentation process to increase the total molar stoichiometry of H_2 production per glucose equivalent close to the theoretical limit of 12. Biological electrochemical systems R&D will lead to environmentally acceptable technology because little metals or toxic materials will be needed and waste biomass or the products of fermentations could provide low cost substrate for the process. Biological fuel cells with robust enzymes might also be used in low power applications in inaccessible areas when sources of organic substrates are available in the environment. The approach could also be applied in the process of wastewater treatment to obtain electricity. The potential for application will be an important objective.

Subtask D will focus on:
i. Microbial fuel cells - coupling microbes to electrodes to produce H_2 from waste biomass.
ii. Enzymatic fuel cells - coupling enzymes to electrodes to produce H_2 from waste biomass.
iii Application of the system for real-world wastewater treatment.

Activity Leader: Alan Guwy (UK)

<u>Subtask E</u>. Overall Analysis

Goal: To determine how to introduce Bio-inspired Hydrogen and BioHydrogen processes as new technologies in support of the coming H_2 society. Adaptation of the technology will be analyzed from the economic, technological, and societal point of view.

Interest in renewable energy technologies such as BioHydrogen has been increasing not only due to their environmentally acceptable characteristics but also to the recent upward trend in the price of oil. Bio-inspired Hydrogen and BioHydrogen are fundamentally different compared to energy systems based on fossil fuels; the energy sources are sunlight and biomass, which are diffuse, but widely available. Therefore, we will have to evaluate how technologies developed under this task will be successfully introduced from sociological and economical points of view. The potential effects of these new technologies on social systems and human life will be evaluated. Analysis of uncontrollable factors (e.g., availability of sunlight and biomass) and risks (e.g., possible release of genetically manipulated microorganisms) should be studied. Other important subjects of interest are economic analyses and social acceptance.

Subtask E will focus on:
i. Effects of Bio-inspired Hydrogen and BioHydrogen on social systems and human life.
ii. Integration of other renewable energy sources with Bio-inspired Hydrogen and BioHydrogen to accelerate practical applications.
iii. Economic and social factors required to implement Bio-inspired Hydrogen and BioHydrogen utilization.

Subtask Leader: Jun Miyake (Japan)

5. <u>Three-Year Work Plans</u> (June 2010- June 2011)

<u>Subtask A</u>

Goals:	December 2010	Selection of components (enzymes, photosynthetic proteins/complexes).
	May 2013	Development of strategies for the construction of bio-inspired systems.

<u>Subtask B</u>

Goals:	December 2010	Assessment and screening of fermentative bacteria.
	May 2013	Characterization of fermentative bacteria and suggestion of target pathways for modification. In integrated systems (dark and photo-fermentation stages), production of at least 6 moles of H_2 per mole of glucose equivalent.

<u>Subtask C</u>

Goals:	December 2010	Analysis of hydrogenase and related genes. Assessment and screening of photofermentative bacteria.
	May 2013	Discovery of the roles of the genes required for the expression of hydrogenases. Improve H_2-production rates in at least one phototrophic microbe. Development of strategies for enhancing the efficiency of light conversion to H_2 in photosynthetic microbes.

<u>Subtask D</u>

Goals:	December 2010	Assessment of suitable organisms.
	May 2013	Development of strategies for the construction of advanced biological fuel cells.

<u>Subtask E</u>

Goals:	December 2010	Identification of the social and economic factors affecting BioHydrogen..
	May 2011	Initiate a case study.
	May 2013	Deliver a case study analysis.

6. Level of Effort

The proposed level of effort under this Task shall be a minimum of 0.5 person year/year (py/y) for each participating country. An additional commitment of 0.3 py/y is required from the Operating Agent, and 0.2 py/y from the Subtask Leaders. Altogether, a minimum of 12 participating researchers supported to specifically carry out the research under the new Task is regarded as necessary to achieve its goals.

7. Participants

Current participants of the Annex are Canada, Finland, France, Germany, Japan, Korea, The Netherlands, Norway, Turkey, Sweden, UK and USA. Poland (in the process of becoming an OECD member), China, Denmark, Italy, New Zealand, Portugal, and Switzerland are in the process of becoming participants or observer countries.

8. Milestones and Deliverables

The major milestones for the Annex are given in Table 1. Regular Task Experts Meetings will be arranged semi-annually. An annual report on the activities and accomplishments of the Annex will be submitted by the OA. An international workshop concerning BioHydrogen production and utilization is planned to be held within the first three years.

9. Communication Plan

Documentation from each subtask and related workshops will be distributed to all participants. Dissemination of related information for industrial and public education will be undertaken. A final report, consolidating the Subtask reports, will be prepared for the Task. For efficient and detailed communication and to accelerate the development of region-specific technologies, Regional Coordinators will be selected from among the experts and will support the Operating Agent.

10. References

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Table 1

Milestones

Subtask A. Bio-inspired Systems.

1) Select stable and efficient components (enzymes, photosynthetic proteins, etc).
2) Promote efficient electron transport between enzymes/photosynthetic proteins and artificial catalyst or chemicals.
3) Examine long term hydrogen production by the systems.

Subtask B. Dark BioHydrogen Systems.

1) Determine yields of anaerobic fermentations as a function of both genetic and environmental determinants with fermentative bacteria.
2) Metabolically engineer fermentative bacteria with improved H_2 production from model substrates and waste waters.
3) Examine thermophilic organisms for increase H_2 production.
4) Examine and optimize an integrated system incorporating a sequential dark anaerobic and photo-fermentation process to enhance H_2-production efficiency from biomass waste.
5) Report on fundamental studies for application to real-life waste treatment.

Subtask C. Basic Studies for Light-driven BioHydrogen Production.

1) Report on the diversity of H_2-production (hydrogenase) genes and their corresponding enzymes in microalgae, cyanobacteria, and other suitable organisms.
2) Determine the relationship(s) among electron transport pathways, carbohydrate catabolism and hydrogenase activities.
3) Improve hydrogen production by genetic engineering, identify salt-tolerant genes, and improve expression of critical genes.
4) Design and test innovative concepts for photobioreactor design and sustained exploitation of the metabolic potential of advanced strains for H_2 production. Demonstrate at the pilot scale (50-100 liters) H_2 photoproduction in an outdoor photobioreactor.
5) Improve the efficiency of light conversion to H_2 in cultures of photosynthetic microbes by optimizing the geometry of the photobioreactors.

Subtask D. Biological Electrochemical Systems.

1) Report on a technological survey of available methods.

2) Improve the growth conditions and power generation of current-generating organisms. Examine the durability and efficiency of operation under various environmental and operating conditions.

3) Determine the potential of and conditions for application to real-life wastewater treatment.

Subtask E. Overall Analysis.

1) Report on societal acceptance of renewable and recyclable energy systems like BioHydrogen.

2) Report on combining Task systems other renewable and recyclable energy systems from an applications point of view, especially for developing countries.

3) Report on cost analyses of Bio-inspired Hydrogen and BioHydrogen production.