TASK 34

BIOLOGICAL HYDROGEN FOR ENERGY AND ENVIRONMENT

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VITAL STATISTICS

Term

2014–2017

Members

Australia, Finland, France, Germany, Italy, Japan, Korea, The Netherlands, Norway, Sweden and UK

Expert Meeting 2014

Malaka, Malaysia 17 December 2014

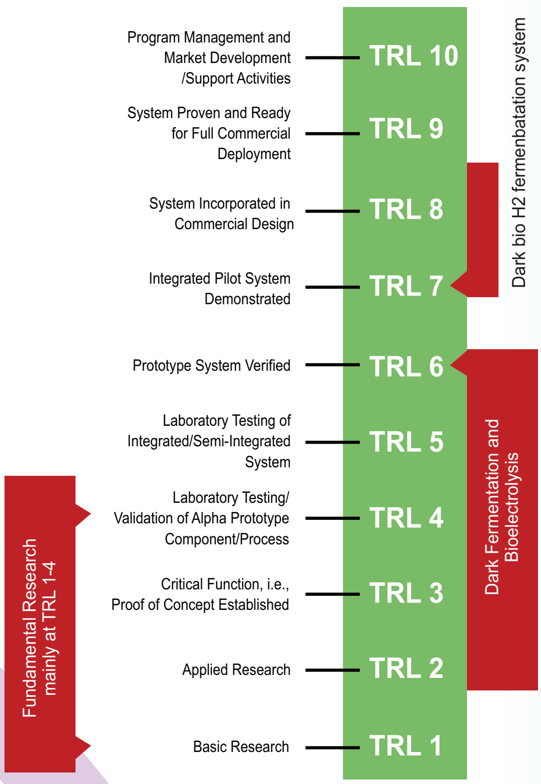
PURPOSE

Task 34 is carrying out collaborative research activities on the biological production of hydrogen using bacterial dark fermentation, photosynthetic microbes and *in vitro* and bio-inspired systems. The overall objective is not only to sufficiently advance the basic and applied science in this area of research over the next three years, but also to evaluate biohydrogen with regards to economics and social impact for both the production of renewable energy and a sustainable environment.

STATUS OF THE TECHNOLOGY

Today, many biohydrogen technologies are being deployed at pilot, demonstration and even at full size. However, there is recognition within the biohydrogen community that the areas of basic research at laboratory scale can and need to be readily transferred to existing pilot and demonstration plants in order to realize the technologies deployment further up the Technology Readiness Level (TRL) scale. Therefore, Task 34 is split into two main subtasks: those that relate to basic research and those that fit into applied research.

Dark fermentation pilot scale systems (TRL 7) have been in operation for a number of years and integration of these systems with existing full scale anaerobic digestion plants is expected within the next five years (TRL 8). Whilst early pilot systems have proven to be successful, the feasibility of integration at commercial scale is likely to depend on work underway in Subtask 1.1 (TRL 2–6). Early uptake at TRL 8 and above of the technology is likely to be influenced by the potential benefits of increased energy production when integrated with existing anaerobic digestion treating waste and agro-industry byproducts. Light-driven biohydrogen energy systems activities concentrate on the basic research aspects. Whilst some work has been successfully scaled up, there is a strong emphasis on fundamental research and the activities are mainly at TRL 1–4.



FRAMEWORK

Task 34 runs from 2014 to 2017 with a new Operating Agent to calibrate two main subtasks that reflect the basic research (Task 1), which continues to be an very active area and applied research (Task 2), which is a growing area of collaboration between industry stakeholders and the university/research institutes involved in the task. Subtask 1 - Basic Research on Biohydrogen Production is subdivided into to 3 subtasks: 1.1: Biohydrogen Production by Dark Fermentation and Bioelectrolysis; 1.2: Light-driven Biohydrogen Production; and 1.3: Enzymatic and Bio-inspired Molecular Systems. Subtask 2 - Applied Research on Biohydrogen Production is subdivided into 3 activities: 2.1: Development and Integration of Biohydrogen Fermentation Systems for Enhanced Energy Production; 2.2: Feasibility of Biohydrogen Energy Systems; and 2.3: Community Based Biohydrogen Systems for Energy Production and Resources.

MEMBERS

Task 34 members are: Australia, Finland, France, Germany, Italy, Japan, Korea, The Netherlands, Norway, Sweden and UK.

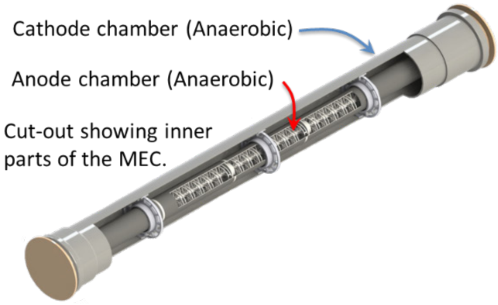
ACTIVITIES AND RESULTS IN 2014

SUBTASK 1 – BASIC RESEARCH ON BIOHYDROGEN PRODUCTION

Subtask 1.1: Biohydrogen Production by Dark Fermentation and Bioelectrolysis

At INRA- Laboratory of Environmental Biotechnology (France), microbial interactions in H_2-producing dark fermentation ecosystems were investigated. Several groups of keystone species were identified as microorganisms having a key role in H_2 producing ecosystems, despite their low relative abundance (<2%). Up to a threefold increase in H_2 production through the use of these species was reported in Nature Communications (Benomar et al. 2015). Research investigations were also carried out on substrate availability with a special attention to H_2 generation. A predictive model was proposed and evaluated suggesting that application of pretreatment was the main factor in increasing H_2 generation (Guo et al. (2014), Motte et al (2014a)).

Membrane bioreactor development was undertaken at GEPEB laboratory Inst. Pascal, France leading to externally-submerged membrane reactor with yields of 1.1 moleH_2 mol^{-1}Glc. A national program DEFIH12 (2009-2013) coordinated by the Laboratory of Chemical Engineering (LGC, Toulouse, FR) aimed at coupling dark fermentation and microbial electrolysis showed that halophilic conditions were favourable for H_2 generation in the MEC. (Pierra et al., 2014). Additionally, a new alkaliphilic *Clostridium* strain was isolated at the Mediterranean institute of Oceanography (Marseille, FR) originating from a submarine hydrothermal chimney (Mei et al, 2014). At the University of South Wales (United Kingdom) controlled, parallel digestion experiments were performed using pelletized grass as a feedstock. These showed that two-stage AD resulted in an increase in energy yields from 10.36 MJ kg-1 VS to 11.74 MJ kg-1 VS, an increase of 13.4% (Massanet et al. 2015). Improvements in digester stability and organic loading rate were also demonstrated. Also at the University of South Wales, conventional electrodialysis has been reported to remove VFAs from hydrogen fermentation broths for the first time (Jones et al. 2015). The ability to remove VFAs from hydrogen fermentation broths has the potential to improve fermentative hydrogen yields. Researchers also investigated the removal of CO_2 and H_2 directly from the headspace of a hydrogen fermentation system increasing H_2 yields from 0.07 to 0.72 mol H_2 mol^{-1} hexose. Researchers are also investigating the integration of fermentative hydrogen production with tubular microbial electrolysis cells to further increase hydrogen yields Boghani et al. 2103, 2014 (Fig. 1).



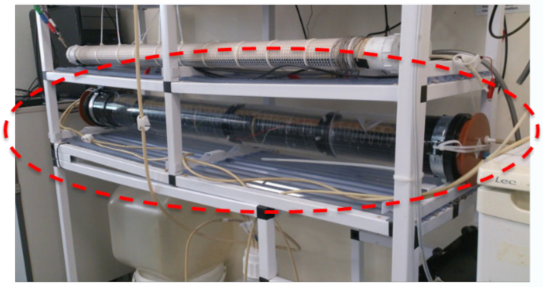


Figure 1. Schematic and photograph of a tubular microbial electrolysis cell for biohydrogen production, developed at the University of South Wales.

Researchers at the Technical University of Denmark are developing an innovative microbial bipolar electrodialysis cell for waste-derived VFA recovery, as well as developing bipolar bioelectrodialysis to recover ammonia and sulfate from waste streams thereby counteracting their toxicity during anaerobic digestion (Zhang and Angelidaki, 2015a, Zhang and Angelidaki, 2015b).

At Wageningen University, hydrogen production from glycerol by hyperthermophilic bacteria and archaea is being studied. By introducing bacterial genes of the oxidative pentose phosphate pathway (OPPP) into the archaeon *Thermococcus kodakarensis,* higher hydrogen levels were obtained and, unexpectedly, the mutant strain also showed a higher growth rate and final cell density.

Subtask 1.2: Light-driven Biohydrogen Production

The indirect pathway of photoproduction has been particularly investigated at the CEA (French Alternative Energies and Atomic Energy Commission). The plastidial type II NAD(P)H was successfully overexpressed in a strain of the microalgae *Chlamydomonas reinhardtii*, with a subsequent increase of hydrogen production rate (Baltz et al., 2014). Also at the CEA overexpression of hydrogenase assembly genes HoxEFUYH and HypABCDE in the model unicellular strain Synechocystis PCC6803 together with an increase of Ni in the medium led to 20-fold increase of the hydrogenase activity, which could lead to increased hydrogen production (Ortega-Ramos, 2014).

At Imperial College (UK), an integrated reactor model was designed to simulate fluid dynamics, local light intensity and the growth rate of nitrogen-fixing cyanobacterium. Recycling gas flow was shown to increase liquid velocity and bubble volume fraction, but prevented light transmission (Zhang et al 2015). Researchers at Imperial have also demonstrated a two-stage aerobic/anaerobic chemostat for the enhanced production of hydrogen and biomass from unicellular nitrogen-fixing cyanobacterium. Compared to a single-stage batch system, the chemostat displayed more than 6.4 times higher H_2 production (Dechatiwongse 2015).

Researchers at the Univeristy of Cambridge (UK) explored the photofermentation of glycerol to hydrogen by *Rhodopseudomonus palustris*, with the objective of maximising hydrogen production. Two piecewise models are designed to simulate the entire growth phase *of R. palustris*; a challenge that few dynamic models can accomplish. By optimising a 30-day industrially relevant batch process, the hydrogen productivity was improved to be 37.7 mL g^{-1} biomass h^{-1} while maintaining the glycerol conversion efficiency at 58%. The University of Warwick in UK studied the location and function of a bidirectional NiFe hydrogenase which transiently produces hydrogen upon exposure of anoxic cells to light using GFP tagging (Burroughs et al. 2014).

Subtask 1.3: Enzymatic and Bio-inspired Molecular Systems

At the Institute for Ecosystem Study, National Research Council (ISE-CNR) researchers have carried out integrated, fundamental research aiming at applying synthetic biology principles towards a cell factory notion in microbial biotechnology. A combination of basic and applied R&D was carried out to design and construct the cyanobacterial cells efficiently evolving and to design and construct advanced photobioreactors that efficiently produce H_2. At the University of Dundee a number of synthetic biology based approaches were investigated to enhance biohydrogen production using *E. coli*. A synthetic (FeFe)-hydrogenase based on a thermostable NADH-dependent hydrogenase was designed, constructed and expressed in *E. coli*. Construction and expression of active H_2-producing synthetic metalloenzymes was evaluated. The bidirectionality of *E. coli* Hyd-2, i.e. H_2 oxidation and H_2 production was demonstrated.

SUBTASK 2 – APPLIED RESEARCH ON BIOHYDROGEN PRODUCTION

Subtask 2.1: Development and Integration of Biohydrogen Fermentation Systems for Enhanced Energy Production

Korea Institute of Ocean Science and Technology, EnsolTek and the Korea Institute of Energy Research have been involved in the development of biohydrogen production technology using the hyperthermophilic archaea; a 12 million USD funded by the ministry of land, transport and maritime affairs. A 300 L of pilot plant was built, and 250 mmol H_2/L fermenter/h of H_2 production rate was achieved by immobilizing hyperthermophilic archaea (*Thermococcus onnurimeus*). A 1 m^3 pilot-plant is now under construction which is connected with coal gasification plant. Wageningen University (NL) co-ordinator of a £3 million project integrating thermophilic dark fermentation with anaerobic digestion. The working volumes of the reactors ranged from 5.8 to 57 and 225 L. For substrates sugars were tested, followed by hydrolysate from verge grass and molasses. A production of 6 g H_2/day using grass hydrolysate and 110 g H_2/day using sugars using has been achieved.

Subtask 2.2: Feasibility of Biohydrogen Energy Systems

The Korea Institute of Energy Research is co-ordinating a 0.5 million USD project on biohythane production from organic solid wastes and business model establishment for rural area application. The main outcome is that from 1 ton of food waste, 60 Nm^3 of biohythane (20% H_2 and 80% CH_4 by volume) has been attained at a 10d HRT. The Université de Rennes (Abdallah et al. (2015)) proposed to add an electrochemical process of nitrate reduction to ammonium that can be further used in dark fermentation process as nitrogen source. This constitutes an interesting alternative for nitrogen supplementation of C-rich biomass with concomitant wastewater treatment.

MEETINGS

Malaka, Malaysia 17th December 2014. Member attendance-Alan Guwy UK , J. Miyake Japan, P. Hallenbeck USA I. OHara Australia, P. Claassen The Netherlands, D.H. Kim Korea. Observer Countries (Taiwan, Thailand, Malaysia).

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