Protective Sensor Coatings for High-Throughput Screening of Hydrogen-Producing Microbes

Hilton G. Pryce Lewis*, Michael Seibert** and David K. Benson***

*GVD Corporation, 45 Spinelli Place, Cambridge, MA 02138, USA, hilton@gvdcorp.com
**National Renewable Energy Laboratory, Golden, CO 80401, USA, mike_seibert@nrel.gov
***Element One, Inc., 7253 Siena Way, Boulder, CO 80301, USA, dkbenson@elem1.com

ABSTRACT

Microbe-based hydrogen production is a potentially cost-effective, non-polluting approach to the production of hydrogen. Research efforts are underway to identify, isolate, and enhance microbial strains which facilitate direct sunlight-to-hydrogen conversion. These efforts are hampered by a lack of adequate instruments to rapidly detect and pinpoint hydrogen producers. There is a need for assays which provide sufficient sensitivity, short response times, scalability, and compatibility with high-throughput methodologies to allow for rapid screening of microbe colonies. GVD Corporation is assisting the National Renewable Energy Laboratory (NREL) in the development of a quick, simple assay that will identify hydrogen producing microbes. This approach could have broader applicability for low-cost hydrogen safety sensors.

Keywords: hydrogen, safety, microbe, sensor, photo-biological

1 INTRODUCTION

Certain types of algae and bacteria can facilitate the conversion of sunlight directly into hydrogen, and this could be the cleanest, most efficient, and most sustainable source of energy for the future. Current research is focused on identifying and isolating organisms that facilitate such processes, understanding the mechanisms by which these work, and amplifying the effect through biological and genetic engineering [1]. The field is growing quickly, but researchers are limited by a lack of adequate methods for detecting and pinpointing hydrogen producers. The principal techniques used to date are Gas Chromatography (GC), Clark Electrodes, commercial hydrogen sensors (e.g. H2Scan) or an indicator solution. Most of these techniques lack one or more of the following characteristics: High sensitivity (0.02% H_2), a rapid response rate (a few seconds), high-throughout or parallel screening capabilities, and an economy in manufacturing that is desirable for rapidly screening colonies of hydrogen-producing organisms on a substrate. Most methods also do not spatially resolve the point where gas is produced in relation to the sample surface, and are therefore not useful in determining the specific location of a colony when the sample consists of many colonies. Finally, typical detection methods rely on electrical signals to determine the presence of hydrogen, which can represent an ignition hazard when large numbers of samples are being tested.

2 SIMPLE, QUICK HYDROGEN ASSAY

GVD is assisting researchers by developing a high-throughput assay (as shown in Figure 1) for assessing and quantifying the production of microbe-based hydrogen in experimental systems [2]. The assay provides:

- Rapid, high-throughput screening capabilities.
- Safety in hydrogen (no electrical circuitry).
- Spatial determination of mutant colonies.
- High sensitivity (~4 nanomoles of hydrogen).
- Reusability and long shelf life.

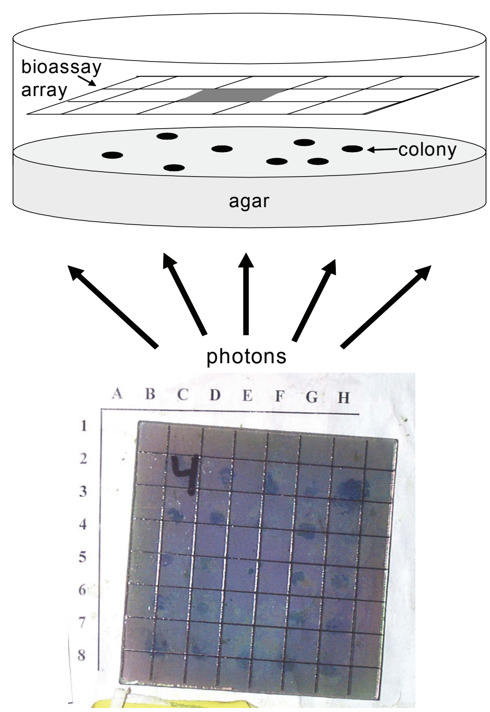


Figure 1: A quick, simple assay for the detection of biologically produced hydrogen.

In its simplest format, the assay consists of a sensor plate which is placed in close proximity to the organisms under investigation. Isolated colonies growing on an agar plate are illuminated by light from below. When hydrogen is produced from a colony, the plate changes color from transparent to dark blue. The position and intensity of the response pinpoints the location of the mutation producing the highest concentration of hydrogen. The plate in Figure 1, for instance, shows a response in blue in a number of locations.

2.1 NREL Sensor Technology

The hydrogen assay is based on a technology developed by the National Renewable Energy Laboratory (NREL) for producing lightweight, low-cost hydrogen sensors. Such sensors allow the monitoring of hydrogen in locations where it is produced, stored, transported, or utilized [3,4]. This new class of sensors displays a chemochromic response in the presence of hydrogen gas. The underlying phenomenon is a chemical reaction between hydrogen atoms and a transparent metal oxide film which produces a color change. In the simplest possible configuration, the color change can be simply observed with the human eye. However, the chemochromic sensors can also be integrated with a light source and an optical detector, even in the compact geometry of a fiber optic system. A schematic of the sensing technology is shown in Figure 2.

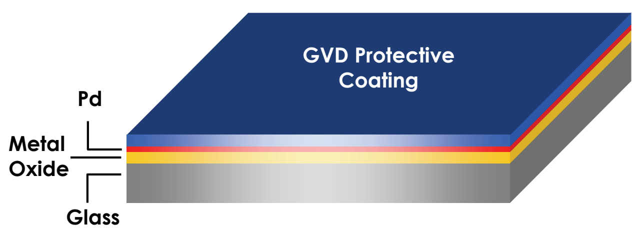


Figure 2: Principle of operation of the hydrogen sensing technology. A chemical reaction between hydrogen atoms and the metal oxide film produces a color change. A protective coating provided by GVD keeps contaminants out and prolongs the life of the sensor.

The assay consists of a series of thin films deposited on a transparent substrate such as glass. Hydrogen diffuses through a protective coating and then dissociates on the Pd layer. Atomic hydrogen travels to the Pd-metal oxide interface and induces a chemochromic effect in the metal oxide. The effect is reversible when hydrogen is removed.

2.2 GVD Protective Coatings

Moisture - in both liquid and vapor form – is a major problem in the warm, humid environments and long timelines required for microbe cultivation. It has a deleterious effect on the assay, causing dead spots in the sensing materials and reducing the reactivity and sensitivity of the assay. GVD provides a protective coating on the sensing materials. This coating enables a durable, robust assay to be produced but does not compromise the inherent sensitivity of the chemochromic mechanism. GVD's coating is also transparent, allowing the chemochromic effect to be observed, and is readily scalable to larger dimensions.

GVD's coating technology is based on chemical vapor deposition, which allows thin, conformal coatings to be produced [5,6]. Importantly, the chemistry and morphology of GVD's coatings can be customized to allow hydrogen gas to reach the sensor materials but prevent atmospheric contaminants and liquid water from doing so. This allows the sensor to achieve maximum sensitivity, short response times, and long lifetimes.

2.3 Assay Formats

Several formats are being developed to address the needs of a diverse set of research protocols. These are shown in Table 1.

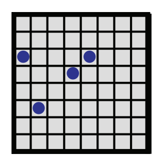
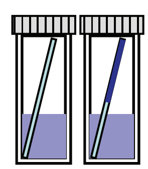
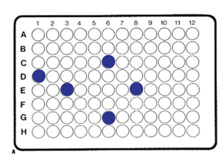
Format	Description
	**Assay Plate** This is an Assay Plate used for the analysis of cultures grown in petri dishes.
	**Anaerobic Vial Assay** This assay is formatted as a dipstick or an insert for vial lids and used to analyze aqueous cultures in vials.
	**Microtiter Plate Assay** This assay consists of an insert which fits into microtiter plate lids to allow for high-throughput screening. Each well can be individually sealed to preserve anaerobicity and prevent bleeding from one well to the next.

Table 1: Assay formats for hydrogen detection

The assay can readily be scaled for larger areas, packaged for PC-based acquisition and analysis, and made available at modest cost to researchers within the community. More complex versions may include automated scanning and measurement capability for even faster measurement and screening.

3 HYDROGEN SAFETY SENSORS

The hydrogen sensor technology described here has application across a range of industries both now and in the future. Current applications include the aerospace industry, where hydrogen is used as a fuel, and remote hydrogen sensing in refineries and chemical plants. GVD is working with several partners to identify applications and commercialize the hydrogen sensor.

In the future, as hydrogen emerges as a mainstream fuel source, sensors will be critical to facilitate situational awareness and rapid response to hydrogen gas leaks. The key performance criteria for hydrogen sensors will be as follows [7]:

- Sensitivity for detecting low levels of hydrogen (<0.5%), well below the explosion limit in air (4% H_2).
- Rapid response (<1 s) so as to provide time for corrective action prior to reaching the explosive limit.
- Reliable response to every incidence of hydrogen exposure coupled with the absence of false alarms.
- Useful lifetime of 6 months to a year in ambient conditions with minimal attention.
- Safe operation, preferably with no potential sources of ignition in contact with hydrogen.

The NREL/GVD sensor can satisfy many of these requirements and will compete well with alternative technologies such as catalytic bead, semiconductor, electrochemical, resistive palladium alloy, and field effect transistor sensors [8]. It has the potential to be less expensive and more portable than many of these approaches. Since it is anticipated that consumers will be reluctant to pay hundreds of dollars per sensing point, it may offer a better solution in consumer-based applications.

For automotive applications such as fuel cell vehicles, for instance, the sensor may have several advantages over other available commercial sensors. In particular, the optical response of the sensor could eliminate the need to locate electrical circuitry close to a hydrogen source. This would both reduce the footprint of the sensor and eliminate a potential source of ignition. The ability to produce the sensor on many different materials and at different sizes could also enable new embodiments of the sensor to be conceived. For instance, both pinpoint sensors (e.g. fiber optic probes) and wide-area safety sensors (e.g. lapel badges) may be required to monitor fuel cell operation and provide constant leak detection.

4 CONCLUSION

A rapid screening method for the detection of hydrogen-producing microbes has been developed. This could be an important enabler for researchers who are seeking to identify microbes which convert sunlight directly into hydrogen. The assay allows for the rapid screening of colonies which are producing hydrogen. As such, it has the potential to allow identification and bioengineering of candidate organisms many times faster than previously possible. Moreover, the technology platform has the potential to address other needs for safety sensors in industries ranging from aerospace to fuel cells.

REFERENCES

[1] M.L. Ghirardi, P.W. King, M.C. Posewitz, P.C. Maness, A. Fedorov, K. Kim, J. Cohen, K. Schulten, and M. Seibert, *Biochem. Soc. Transact.* 33, 70–72 (2005).

[2] M. Seibert, D.K. Benson, T.M. Flynn, US Patent Nos. 6,448,068 (2002) and 6,277,589 (2001).

[3] S.H. Lee, E.C. Tracy, J.R. Pitts, P. Liu, US Patent No. 6,723,566 (2004).

[4] R.D. Smith, P. Liu, S. H. Lee, C. Tracy, J. R. Pitts *Fuel Chemistry Division Reprints* 47, 825 (2002)

[5] K.K. Gleason, S.J. Limb, E.F. Gleason, H.H. Sawin, D.J. Edell, US Patent Nos. 5,888,591, 6,153,269, 6,156,435, (1999, 2000).

[6] K.K.S. Lau, S.K. Murthy, H.G. Pryce Lewis, J.A. Caulfield, K.K. Gleason, *J. Fluor. Chem.* 2003, 122, 93.

[7] R.D. Smith, P. Liu, S.H. Lee, E. Tracy, J.R. Pitts. *Proceedings of the 2001 US DOE Hydrogen Program Review* (2001)

[8] A. P. Jardine, *Power Pulse*, 11/13/2000.

`