Available online at www.sciencedirect.com
SCIENCE DIRECT
International Journal of Hydrogen Energy 31 (2006) 1490–1495
International Journal of HYDROGEN ENERGY
www.elsevier.com/locate/ijhydene

Integration of biohydrogen fermentation and gas separation processes to recover and enrich hydrogen

K. Bélafi-Bakó[a,*], D. Búcsú[a], Z. Pientka[b], B. Bálint[c], Z. Herbel[c], K.L. Kovács[c], M. Wessling[d]

[a]*Research Institute of Chemical and Process Engineering, University of Veszprém, Egyetem u. 2., 8200 Veszprém, Hungary*
[b]*Institute of Macromolecular Chemistry, Czech Academy of Sciences, Heyrovsky sq. 2., Prague, Czech Republic*
[c]*Department of Biotechnology and Institute of Biophysics, Biological Research Center, Hungarian Academy of Sciences, University of Szeged, Temesvári krt. 62., 6726 Szeged, Hungary*
[d]*Membrane Technology Group, University of Twente, P.O. Box 217, 7500 AE Enschede, The Netherlands*

Available online 18 July 2006

Abstract

An integrated system for biohydrogen production and separation was designed, constructed and operated where biohydrogen was fermented by *Thermococcus litoralis*, a heterotrophic archaebacterium, and a two-step gas separation process was coupled to recover and concentrate hydrogen. A special liquid seal system was built to deliver, compress and collect the laboratory scale, low volume gas mixtures consisting of hydrogen, nitrogen and carbon dioxide. As a result, gas mixture with 73% high hydrogen content was produced by a combination of a porous and a non-porous gas separation membrane.

Keywords: Gas separation; Integrated system; Polymer membranes; Biohydrogen

1. Introduction

Hydrogen—as a promising alternative energy carrier ("clean fuel")—can be produced in several ways, including environment-friendly methods such as biological systems [1]. Methods for biohydrogen production can be divided into two main groups. During biophotolysis hydrogen is produced from water by using (sun)light. The other method is the so called "dark" fermentation [2], where hydrogen production is carried out by heterotrophic, anaerobic microorganisms. The energy necessary for the operation of the biological system is provided by organic substances.

The gas mixture—containing hydrogen—obtained in the fermentation process is not suitable for direct utilization. Partly because the concentration of hydrogen is not high enough for applications (e.g. fuel cell), and partly because other gases (e.g. carbon dioxide) formed as a result of the biological activity may interfere with the applications. Continuous removal of hydrogen is extremely important for the potential continuous mode of operation of such systems, particularly because hydrogen accumulation generally reduces the hydrogen production rate during the fermentation.

One of the serious problems in hydrogen fermentation is the well known product inhibition phenomenon

* Corresponding author. Tel.: +36 88 624 044; fax: +36 88 624 038.
E-mail address: bako@mukki.richem.hu (K. Bélafi-Bakó).

0360-3199/$30.00
doi:10.1016/j.ijhydene.2006.06.022

(growing H_2 partial pressure in the head space). Thus, separation of hydrogen is not only important for the transport, storage and end use, but also to ensure the sustained biohydrogen production. The concentration of biohydrogen in the headspace of the fermentor depends on the volume ratio of the headspace and the broth, but is usually not high enough for direct usage, therefore, it should be enriched.

Microbial hydrogen-producing systems work usually under anaerobic conditions in closed vessels, where inert atmosphere is provided by nitrogen or argon gas. Therefore, separation of hydrogen from the inert gas is essential. Moreover, the other gases produced (e.g. CO_2) should be separated from hydrogen, as well.

There are several, potentially applicable methods for hydrogen separation and concentration using membrane processes alone [3], or combined with other methods, e.g. electrochemical techniques [4]. Gas separation is one of the most often used membrane techniques [5]. Gas separation is a pressure driven membrane operation where gas mixtures are separated by porous or non-porous membranes. It can be characterized by permeability and selectivity. Permeability can be determined for single gases experimentally from the permeances. Permeance values are measured in $cm^3\ min^{-1}$, then expressed in gas permeation unit (GPU), where $1\,GPU = 10^{-6}\,cm^3$ (STP) $cm^{-2}\,s^{-1}\,Hg\,cm^{-1}$. Permeability coefficients can be calculated from the permeability and the top layer thickness, and often expressed in Barrer ($1\,Barrer = 10^{-10}\,cm^3$ (STP) $cm\,cm^{-2}\,s^{-1}\,Hg\,cm^{-1}$). The selectivity of the membrane for a two-component mixture is defined as the ratio of the permeance values (or permeability coefficients) [3,5].

Hydrogen separation has been applied so far mainly in catalytic (de)hydrogenation reaction at elevated temperatures [6,7]. Therefore, highly thermostable membranes were used, made from expensive inorganic materials (e.g. palladium). Separation of biohydrogen, however, does not require high temperature conditions, thus less thermostable (and cheaper) polymer membranes—having proper hydrogen selectivity—may be suitable. Among the non-porous gas separation membranes, poly(vinyl-chloride), poly(ether-ketones), polyimides and polyether–sulphone–polyimide composite membranes have quite high membrane permeability for hydrogen at 25 °C: 14 Barrer [5], 11.6–36.1 Barrer [8], 8.3–23.1 Barrer [9] and 4.6 Barrer [10], respectively. Moreover, these membranes show good selectivity in N_2–H_2 separation, as well.

Although hydrogen separation has been studied for long, only a few papers have been published so far on the application of the membrane techniques to recover biohydrogen from the biological system [11,12]. Liang et al. applied e.g. silicone rubber membrane to separate biohydrogen from the liquid phase [11], while membrane contactors were used by Teplyakov et al. [12]. However, these studies focused onto the CO_2–H_2 separation, N_2 separation has not been mentioned. In anaerobic hydrogen fermentations N_2 is usually used as inert gas, therefore it should be separated when production of pure H_2 is aimed.

Porous membranes may be suitable for certain gas separations based on Knudsen-flow [3]. In these cases permeabilities of the gases are influenced only by their molecular weight and separation is determined by the ratio of the gases' molecular weights.

Gas separation is traditionally carried out by using pumps and compressors at the feed side and permeate is obtained at the secondary side using vacuum pumps [13]. To operate these gas separation systems (pumps and compressors) at least 1–2 $dm^3\ min^{-1}$ flow rate is required. During recovery of permeates through the vacuum pump, the volume of the permeate should be larger at least by one order of magnitude than the dead space of the equipment, otherwise permeate cannot be collected.

In laboratory scale biohydrogen fermentation, the volume of the hydrogen containing gaseous mixture product is usually much smaller than that is required for the operation of classical gas separations, so these systems are not directly suitable for biohydrogen recovery. Therefore, a novel and innovative system is needed for delivery, compressing and collection of gaseous mixtures and gas separation modules should be built in.

The aim of this study was to couple the fermenter and the gas separation unit(s) to recover and concentrate biohydrogen by using a special liquid seal system to adjust and control pressure, moreover to deliver and collect the gas mixtures (feed, permeate, retentate). In the fermenter biohydrogen was produced by *Thermococcus litoralis,* a heterotrophic, anaerobic microorganism. To the best of our knowledge, no laboratory scale system has been constructed so far, where the combination of biohydrogen fermentation and membrane gas separation process for hydrogen recovery was applied.

2. Experimental

2.1. Materials

The initial materials for non-porous membrane were Matrimid 5218 polyimid (Ciba-Geigy) and Sumikaexcel polyether–sulfone (Sumimoto). The polyimid consisted of 3,3′,4,4′-benzophenon tetracarboxyl dianhydrid and diamino-phenilidene. The substances for the fermentation and analysis were analytical grade and

purchased from Sigma. The H_2, N_2 and CO_2 gases in cylinders were the products of Messer Hungariagas (Hungary).

2.2. Fermentation

Thermococcus litoralis, a hyperthermophilic, heterotrophic microorganism was grown on a complex medium to produce biohydrogen [14]. One litre of this broth contained the following components: 24 g NaCl, 10.6 g $MgCl_2 \times 6H_2O$, 4 g Na_2SO_4, 1.5 g $CaCl_2 \times 2H_2O$, 0.7 g KCl, 0.2 g $NaHCO_3$, 0.1 g KBr, 0.025 g $SrCl_2$, 0.03 g H_3BO_3, 0.2 mg resazurin, 1 g yeast extract, 5 g peptone; pH adjusted to 6.5; heat-sterilized; after cooling it was supplemented with 1.3 ml of filter sterilized 15% cysteine—HCl solution. Alternatively, the mineral medium was supplemented with hydrolysed keratine-rich biowaste (feather meal, containing various amount of amino acids [14]) as the organic substrate of the fermentation. Fermentation was initiated by adding 1% inoculum, and it was carried out under nitrogen atmosphere at 85 °C temperature. Cell growth was followed by OD measurement, while gas composition of the headspace was determined by gas chromatography (see below).

2.3. Non-porous membrane

The PES/PI non-porous hollow fibre membranes were built into a stainless steel tube. The membrane surface area was 12 cm^2. Gas mixture was fed into the shell side of the module by using 400–500 kPa pressure. The module was placed into a glass-walled thermostat and 50 °C temperature was maintained.

2.4. Porous membrane

Porous membrane (pore size 0.05 μm) was fabricated from high density polyethylene (HDPE) as hollow fibres, inner diameter was 0.2 mm. The hollow fibres were built in a module by bending and sealing (by a two-component glue) both ends into one hole. The membrane surface area was 5 cm^2.

2.5. Samples and analysis

Samples were taken from the headspace of the fermenter, from the feeds, retentates and permeates by a gas-tight syringe in duplicate. The composition of the samples were determined by gas chromatography using Hewlett Packard 5890 Series II equipment, Carbonplot column, TCD detector, HP 3394 A integrator and nitrogen as carrier. Calibration with H_2 and CO_2 pure gases was performed prior to the measurements.

3. Results

During the fermentation a three-component gaseous mixture is present in the fermenter headspace, in addition to water vapour, consisting of 20–25% hydrogen and 10–15% CO_2, the remaining is nitrogen, —according to the fermentation measurements [14]. It seemed too difficult to separate biohydrogen in one single step from N_2 and CO_2 simultaneously, therefore a two-step gas separation system was designed, where H_2–N_2 and H_2–CO_2 separations were carried out, by non-porous and porous membranes, respectively.

3.1. Testing of the non-porous membrane

In the first step separation of H_2 and N_2 was the goal. PI-PES hollow fibre membrane module was tested with pure hydrogen and nitrogen and the permeability coefficients were found as 4.6 and 0.11 Barrer, respectively [15]. Thus, the selectivity (42) seemed high enough for hydrogen separation.

Further experiments were carried out with the non-porous membrane module under various conditions to determine the optimal operating parameters. Regarding transmembrane pressure (TMP), it seemed that at least 300 kPa TMP was required for the successful operation. Investigating the effect of temperature, we found that higher permeabilities could be achieved at elevated temperature, and the selectivity increased up to 50 °C as well. Moreover, it turned out that water vapour had a disadvantageous effect on H_2 permeability and selectivity (Fig. 1). Therefore, water had to be removed before the membrane module by e.g. a silica trap.

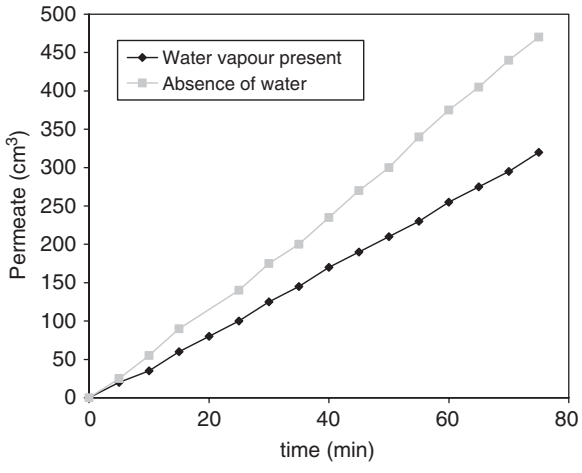

Fig. 1. The effect of water vapour on the permeability of hydrogen.

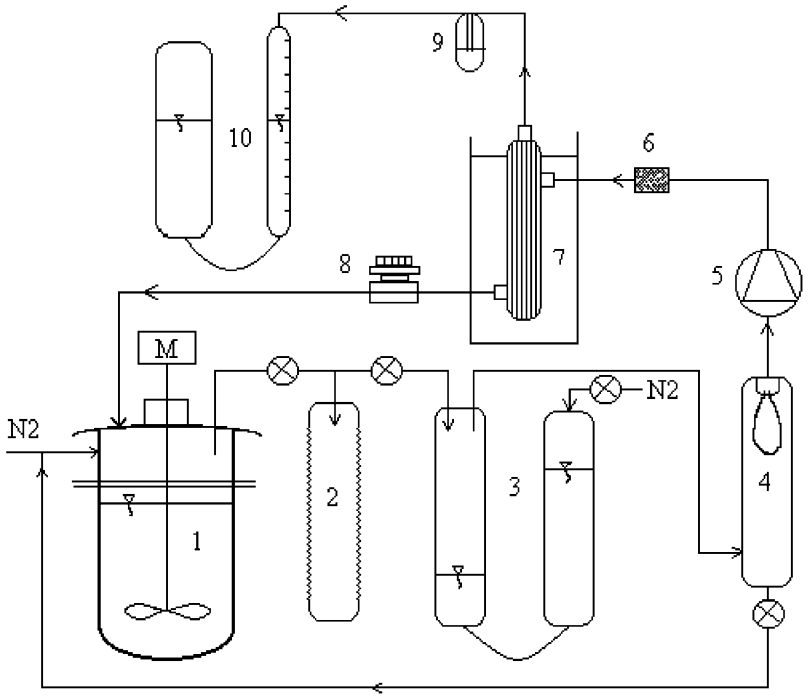

Fig. 2. Scheme of the integrated system: (1) fermenter; (2) elastic-wall buffer tank; (3) gas reservoir with liquid sealing; (4) porous membrane module; (5) peristaltic pump; (6) silica gel trap; (7) non-porous gas separation module; (8) valve for pressure control; (9) bubble-counting tube; (10) gas burette to collect hydrogen.

3.2. Testing of the porous membrane

Separation of H_2 and CO_2 was studied by porous membranes, where separation is based on the Knudsen-flow. Theoretically, the maximal separation factor is 4.7. The bent, porous hollow fibres were placed directly into the feed gaseous mixture and the lumens were connected to a peristaltic pump, which sucked the gases through the membrane wall by creating 10–50 kPa vacuum in the lumen side. Thus, the permeate became rich in H_2 and poor in CO_2, while the retentate displayed higher concentration of CO_2. The selectivity achieved in the measurements was 4.0.

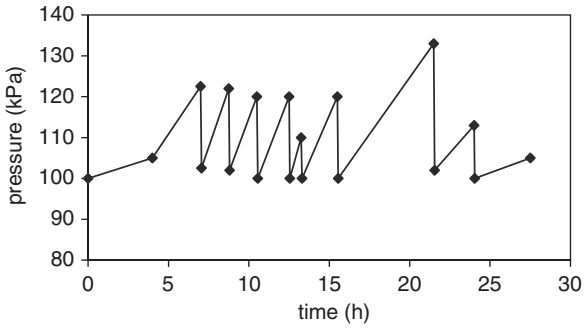

Fig. 3. Pressure data in the headspace of the fermenter during the fermentation.

3.3. Integrated system

To design a suitable system for the biohydrogen separation numerous criteria had to be considered. The gaseous mixture above the broth should be removed without disturbing and contaminating the fermentation process. This could be achieved by an elastic-wall reservoir through an aseptic syringe, controlling the recovery process by a liquid seal system. The mixture was designed to separate first by the porous membrane (applying low vacuum using a peristaltic pump) to reduce the CO_2 content. Then the gas mixture had to be compressed in order to feed into the non-porous membrane module, so a throttle valve was built in. The permeate—rich in H_2—could be recovered simply in a burette under atmospheric pressure, which was controlled by liquid seal system, as well.

The scheme of the integrated system is depicted in Fig. 2. The liquid seal reservoirs were made of thick glass wall, the liquid used was water. Pressure was

Table 1
Permeation data for biohydrogen recovery by two-stage membrane separation

Exp.	Feed gas mixture (from fermenter)		Permeate of porous membrane		Permeate and retentate of non-porous membrane			
	Volume (cm^3)	H_2 content (%)	Volume (cm^3)	H_2 content (%)	Volume (cm^3)	H_2 content (%)	Volume (cm^3)	H_2 content (%)
No. 1	875	25	345	39	51	75	294	29
No. 2	1020	21	375	36	68	71	307	26
Average	—	23	—	37.5	—	73	—	27.5

adjusted and maintained through the water seals by nitrogen gas (from cylinder) and by a peristaltic pump. To follow the collection of hydrogen rich permeate, a small bubble-counting tube was built in. Before the non-porous membrane module a silica trap for water vapour removal was placed. Small size manometers and vaco-manometers were used to measure pressure. Connecting tubes were made of gas tight copper, joint pieces were made of copper and teflon.

First the whole separation system was checked experimentally to test if it was pressure-tight. Following the successful tests it was connected to the biological fermenter system under sterile conditions. During the fermentation the first 4–5 h (Fig. 3) was the so-called "lag" period, where microbial growth occurred, and no hydrogen was formed. Then biohydrogen production started, which was indicated by the continuously growing pressure in the headspace. (The strain releases CO_2 beside hydrogen.) The pressure in the fermentor was controlled by removing the gas mixture from the fermentor head space regularly (maintaining the pressure between 100 and 130 kPa). During the experiments gas mixture removal was usually scheduled when pressure reached 120 kPa, but we tried to vary it: scheduling gas removal by 110 as well as 130 kPa to see if there was any effect on the hydrogen formation. On the other hand the pressure in the fermenter was not allowed to decrease below 100 kPa during removal of gases, otherwise the broth might start to boil (under vacuum, 85 °C). Finally, biohydrogen formation slowed down after 20 h and stopped. The figure shows *one* time course (a particular fermentation) as an example, but several similar experiments were carried out, resulting in similar data.

The gas mixture was collected in an elastic-wall reservoir which served as a buffer tank and the components were separated by the two-step membrane system. In the first step H_2 was purified on the porous polymer membrane, the resulting permeate was poorer in CO_2. It was then pumped into the shell side of the non-porous hollow fibre membrane by a peristaltic pump through a throttle valve to increase the pressure to 500 kPa. Water content of the gas mixture was removed by the silica trap. Permeation data are summarized in Table 1. The collected gas mixture was separated in two (parallel) experiments and the volumes as well as H_2 contents of the feed, retentate and permeates of the porous and non-porous membrane units are listed in Table 1. The permeate obtained had 73% hydrogen content (average) which seems high enough for utilization. It should be noted that considerable amount of H_2 was being lost in the concentration process. To decrease these losses the operation of the two-stage separation system should be optimized.

4. Conclusions

The experiments described above demonstrated that it is possible to recover and enrich hydrogen as a complex gas mixture in a biotechnological process. The porous and non-porous membrane gas separation units with built in liquid seal system assembly formed an integrated system and provided a gas mixture as a permeate, containing over 70% hydrogen.

The system, however, requires further improvement before large scale use. The retentate obtained in the first step (porous membrane) is rich in CO_2, which has a disadvantageous effect on the separation if it is operated in a recirculation cycle, since CO_2 content is gradually increasing. Therefore, another step should be built in the system for removal of CO_2. It can be achieved by e.g. gas–liquid absorption where membranes can be used, as well (i.e. contactors).

During the measurements in the integrated system, gases formed in the closed fermenter increased the pressure above the broth, thus partial pressure of H_2 became gradually higher, as well. This phenomenon is unfavourable for the H_2 production of the microbes due to product inhibition, so removal of the gas mixture regularly from the fermenter has a beneficial effect on the H_2 formation process, too. On line gas treatment and semi-continuous operation therefore allows longer operational time and improved hydrogen productivity in the biological system.

In general terms, we believe that the system developed for the present biotechnological application can be applied in the biohydrogen fermentations by other microbes, which may produce gas mixtures of distinct composition. A scale-up of the laboratory system is planned based on the experiences obtained in near future.

Acknowledgements

The research work was supported by a Hungarian Ministry of Education project (2002–2004), Grant no. BIO 00109/02 to KLK and BBK, a Békésy György Postdoctoral Scholarship (2002–2005), Grant no. 20/2002 to BBK and the Bilateral Research Programme between Czech and Hungarian Academies of Sciences (2004–2006) to BBK and PZ. BB and KLK thanks for the financial support from EU 6th FP STREP-SOLAR-H and HyVolution and GVOP-3.1.1.-2004-05-0136/3.0.

References

[1] Benemann J. Hydrogen biotechnology: progress and prospects. Nature Biotechnol 1996;14:1101–3.
[2] Nath K, Das D. Improvement of fermentative hydrogen production: various approaches. Appl Microbiol Biotechnol 2004;65:520–9.
[3] Mulder MHV. Basic principles of membrane technology. Dordrecht: Kluwer Academic Publishers; 1996.
[4] Lee HK, Choi HY, Choi JH, Park JH, Lee TH. Hydrogen separation using electrochemical method. J Power Sources 2004;132:92–8.
[5] Toshima N (Ed.). Polymers for gas separation. New York: VCH Publishers, 1992.
[6] Elkina IB, Meldon JH. Hydrogen transport in palladium membranes. Desalination 2002;147:445–8.
[7] Morreale BD, Ciocco MV, Enick RM, Morsi BI, Howard BH, Cugini AV, Rothenberger KS. The permeability of hydrogen in bulk palladium at elevated temperatures and pressures. J Membr Sci 2003;212:87–97.
[8] Maier G, Wolf M, Bleha M, Pientka Z. Gas permeabilities of polymers with indan groups in the main chain. 1: poly(ether ketone)s. J Membr Sci 1998;143:105–13.
[9] Maier G, Wolf M, Bleha M, Pientka Z. Gas permeabilities of polymers with indan groups in the main chain. 2: polyimides. J Membr Sci 1998;143:115–23.
[10] Kapantaidakis GC, Koops GH. High flux polyethersulfone—polyimide blend hollow fibre membranes for gas separation. J Membr Sci 2002;204:153–71.
[11] Liang TM, Cheng SS, Wu KL. Behavioral study on hydrogen fermentation reactor installed with silicone rubber membrane. Int J Hydrogen Energy 2002;27:1157–65.
[12] Teplyakov VV, Gassanova LG, Sostina EG, Slepova EV, Modigell M, Netrusov AI. Lab-scale bioreactor integrated with active membrane system for hydrogen production: experience and prospects. Int J Hydrogen Energy 2002;27:1149–55.
[13] Whyte NM (Ed.). Industrial gas separations. ACS symposium series. Washington: American Chemical Society, 1983.
[14] Bálint B, Bagi Z, Tóth A, Rákhely G, Perei K, Kovács KL. Utilization of keratin-containing biowaste to produce biohydrogen. Appl Microbiol Biotechnol 2005;69:404–10.
[15] Horváth R, Orosz T, Bálint B, Wessling M, Koops GH, Kapantaidakis GC, Bélafi-Bakó K. Application of gas separation to revocer biohydrogen produced by *Thiocapsa roseopersicina*. Desalination 2004;163:261–5.