Desalination and Water Treatment
www.deswater.com

14 (2010) 252–258
February

1944-3994/1944-3986
doi: 10.5004/dwt.2010.1104

Membrane-assisted separation of microbial gaseous fuels from renewable sources

A. Netrusov[a*], S. Abramov[a], E. Sadraddinova[a], A. Shestakov[a], M. Shalygin[b], V. Teplyakov[b]

[a]*Microbiology Department, Moscow State University, Moscow 119992, Russia*
Tel. +7 495 939 2763; Fax.: +7-495-939-27-63; email: anetrusov@mail.ru
[b]*A.V. Topchiev Institute of Petrochemical Synthesis, Russian Academy of Science, Moscow 119991, Russia*

Received 31 July 2009; accepted 22 December 2009

ABSTRACT

One possible way to obtain fuel gases is using of bioreactors running on cellulose and producing H_2/CO_2 gas mixtures. This method has many advantages, for example: high ecological efficiency, utilization of organic wastes, low energy consumption, accessibility and simplicity of hardware implementation. The results of cellulose bioconversion into hydrogen by using an active membrane, system integrated with anaerobic thermophilic bioreactors, are presented. The suggested membrane system includes anaerobic hydrogen bioreactor and membrane module. Fermentation broth with dissolved gases was transferred into membrane module, where gases were separated from fermentation broth with its circulation back into anaerobic bioreactor. The developed system helps to obtain energy from organic substrates in the form of pure combustive gas (H_2).

Keywords: Membrane module; Gas isolation; Anaerobic bioreactor; Thermophilic cellulose decomposition; Hydrogen

1. Introduction

At present time serious problem exists with processing and utilization of organic waste, especially from large cities. All urban solid wastes are now depositing in large landfills, creating problems with land use, pollution of subsurface waters and malodor. According to official data, metropolis like Moscow may create as much as 19 million tons of solid wastes a year and more then quarter of it is an organic waste [1]. Therefore the process of ecological friendly utilization of organic waste has a great significance. In the view of renewable energy obtaining, the development of the process for fuel gases production from organic wastes has also a priority.

Hydrogen is the one of the most ecological pure renewable fuel. It has high combustion value and upon firing do not evolve greenhouse gases. Anaerobic decomposition of organic raw materials with thermophilic microorganisms is one of the most perspective method for hydrogen obtaining from organic waste [2,3]. However, technology of subsequent hydrogen processing has some essential imperfections. Evolved hydrogen usually polluted with other gases (CO_2, H_2S) and is in low volumetric concentration. Hydrogen in gas mixture does not allow to apply it directly for the fuel cell. Moreover, one more serious obstacle also exists. High partial pressure of hydrogen in headspace severe inhibits fermentation process itself [4]. This problem usually

*Corresponding author

Presented at the Fourth Membrane Science and Technology Conference of Visegrad Countries (PERMEA 2009), Prague, 7-11 June, 2009.

could be solved by mean of extraction of hydrogen from fermentation broth. Different techniques such as sparging, flushing with inert gases, vacuumming of fermentation broth and others are used for this purpose [5–8].

Membrane technologies exist, which were successfully used for gases separations. The porous membranes have a particular wide distribution. However, porous membranes have some imperfections which do not allow to use them for microbiological processes. They not guarantee sterility, have low selectivity and are subjected for biofouling, thus can bring the whole process to stoppage. Therefore it is preferably to utilize non-porous membranes [9] in combination with bioprocesses.

A process of hydrogen extraction from fermentation broth using non-porous membranes based on polyvinyltrimethylsilane (PVTMS) was developed in this work. PVTMS membranes were widely used due to high permeability and suitability for a biotechnology [10–14].

The goals of this work were the screening of thermophilic associations of microorganisms which are able to decompose cellulose and produce hydrogen, the creation laboratory-scale membrane fermentor for conversion of raw organic materials to hydrogen and in application of membrane technologies in gas separation process using submerged membranes working at high temperature.

2. Materials and methods

2.1. Organisms and growth medium

Imshenetskij's medium (medium 1) for anaerobe cellulolytic microorganisms isolation consist of (g/L): $NaNH_4PO_4$ –- 1.5; K_2HPO_4 – 0.5; KH_2PO_4 – 0.5; $MgSO_4 * 7H_2O$ – 0.4; NaCl – 0.1; $MnSO_4 * 4H_2O$ – trace; $Fe\ SO_4 * 7H_2O$ – trace; $CaCO_3$ – 2.0; peptone – 5.0. The pH of the medium was 7.0–7.2 before autoclaving [15]. As an organic substrates were used: paper (filter paper, newsprint, magazine paper, 15.0 g/L), cellobiose (7.5 g/L), wheat bran (10.0 g/L), wood's sawdust (15.0 g/L), glucose (5.0 g/L). For visual confirmation of anaerobiosis in the medium resazurin was added at 0.5 mg/L. DSM medium (medium 2) [16] for cellulosolytic microorganisms cultivation consists of (g/L): NH_4Cl – 0.90; NaCl – 0.90; $MgCl_2 \times 6\ H_2O$ – 0.40; KH_2PO4 – 0.75; K_2HPO_4 – 1.50; trypticase – 2.0; yeast extract – 1.0; trace element solution SL-10 – 1.0 ml; $FeCl_3 \times 6\ H_2O$ – 2.50 g; cellobiose or cellulose – 1.0; cysteine-HCl $\times$ H_2O – 0.75; resazurin – 0.5 mg; distilled water up to 1,000.0 ml; pH was adjusted to 7.2. Media were prepared anaerobically under 100% Ar and autoclaved at 0.5 atm in rubber-stoppered flaks (volume – 450 ml with 100 ml of medium in it). Gas phase in the flasks was Ar.

Samples for inoculations were collected from different natural niches such as soil, silt, decomposed leafs, freshwater mud, animal wastes. Media were inoculated under sterile Ar flow with about 10% of inoculums of the media volume. Cultivations for enrichment cultures were conducted on shaker at 60°C and 70°C (at 35 rpm).

2.2. Estimation of metabolic products

Gas phase analysis were performed at 120°C with a Crystal 5000.1 chromatograph (Russia), equipped with 10 m $\times$ 0.5 mm activated carbon column with argon as a carrier gas and catarometer as a detector. The GC signals were integrated with a Chromatec Analytic 2.5 computer program (Russia).

2.3. Determination of volatile fatty acids

Analysis of the cellulose degradation liquid products were performed by GLC, using a Crystal 5000.1 chromatograph, equipped with a column 10 m $\times$ 0.5 mm with helium as a carrier gas, FID and the temperature gradient was from 60 to 160°C. Results of chromatography were integrated with a Chromatec Analytic 2.5 program. Glucose was measured by glucose strip test (with glucose oxidase). Morphology of cells was examined under electronic microscope JEOL JSM-6380LA (Japan).

2.4. Polymeric membranes

Three dense membranes were selected as candidates for application in membrane bioreactor: industrial composite membrane based on poly-dimethylsiloxane (PDMS); industrial asymmetric membrane based on poly-vinyltrimethylsilane (PVTMS); laboratory composite membrane based on poly-trimethylsilylpropyne (PTMSP). The preparation of PTMSP membrane was carried out by coating of ultrafiltration membrane by the solution of PTMSP in toluene. After coating membrane was dried on the air for 24 h at room temperature.

The permeance of the membranes were tested for pure gases by standard measurement technique: gas flow rate was estimated at given transmembrane pressure drop (Fig. 1). Membrane permeance was calculated as following:

$$Q = \frac{J}{A\Delta p}$$

where Q is permeance [$L\cdot(m^2)^{-1}\ h^{-1}\ bar^{-1}$], J is a gas flow rate [$L\ h^{-1}$], A is membrane area [m^2] and Δp is transmembrane pressure drop [bar].

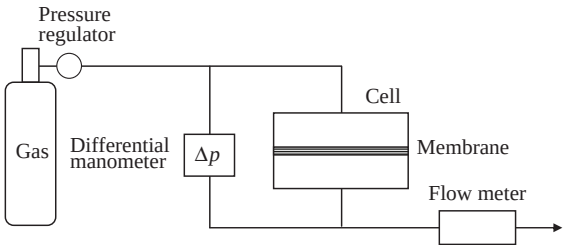

Fig. 1. The scheme of the setup for membrane permeance measurement.

3. Results and discussion

3.1. Experimental setup

For the experimental work on the hydrogen removal from the cultural media and for membrane module construction several non-porous membranes were selected and tested. Results of the permeability tests are shown in Table 1. PVTMS membrane was selected for the further work.

PVTMS membranes are produced by phase inversion method and consist of thin nonporous selective layer (~2μm) and porous support layer (120-150 μm). These membranes were selected due to their high permeance, industrial origin and already demonstrated suitability for application in biotechnology [Table 1; 10–14]

The possibility of PVTMS membranes biofouling was investigated. The membranes were submerged into anaerobic rubber-stoppered flasks with active cellulose-degrading consortia. Incubation in the flasks was four weeks. After incubation scanning electronic microscopy (SEM) of membrane surfaces was performed (Fig. 1). Investigation of membrane surface did not show the signs of biofouling. Only very few cells situated far from each other were found on the surface of the membrane and the formation of biofilm was not observed. The permeance of membrane did not change after four weeks of incubation in flask with microbial consortia. Some cracks which can be seen on the membrane surface (Fig. 2) are the result of the fast drying method of sample preparation for SEM.

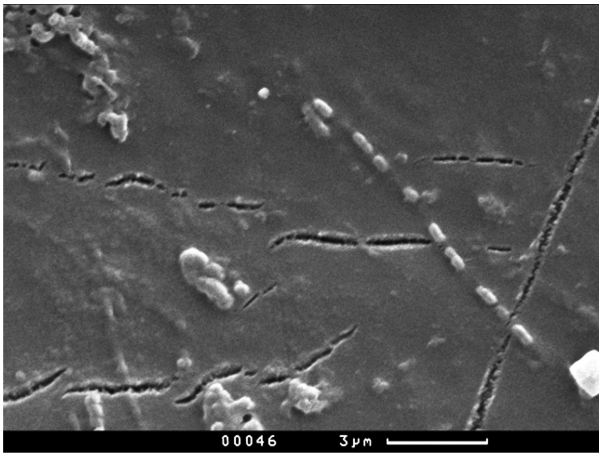

Fig. 2. Electronic microphotography of PVTMS membrane surface after 4 weeks of incubation in the medium with active microbial consortia, degrading cellulose.

On basis of PVTMS membranes a membrane module was constructed (Fig. 3) consisting of four double-sided membrane cartridges mounted into plastic container where gas was recovered from liquid medium flow (Fig. 4). The volume of plastic container was 800 mL. Membrane cartridges were fastened on central Plexiglas tube. The internal space of this tube was connected with internal spaces of four membrane cartridges through aperture which was cut out along the tube. Membrane packages were separated by rubber and plastic gaskets. Such construction allows to secure a pressure-tight joint between internal space of metal tube and four cartridges. Diameter of each cartridge was 120 mm, membranes were arranged on porous plastic support. Area of one membrane cartridge was 0.044 m^2. The maximal theoretical permeabilities for H_2 and CO_2 by this area were 290.3 L h^{-1} bar^{-1} and 280.7 L h^{-1} bar^{-1} accordingly (Fig. 4).

The membrane module was integrated with laboratory-scale reactor (1.5 L) designed for sterile microbial conversion of organic raw materials into hydrogen. General view of laboratory-scale reactor is shown in the Fig. 5.

Medium from anaerobic reactor (Fig. 5, 1) was constantly transferred to membrane module (Fig. 5, 2) by

Table 1
Gas transfer characteristics of selected non-porous polymeric membranes

Polymer	Thickness of dense layer, μm	Permeance of membrane, [Q, L·(m^2)$^{-1}$·h^{-1}·bar^{-1}]				
		CH_4	CO_2	H_2	N_2	O_2
Polydimethylsiloxane (PDMS)	1.5	230	1000	200	90	170
Polyvinyltrimethylsilane (PVTMS)	0.2	200	1600	1700	120	450
Polytrimethylsilylpropyne (PTMSP)	5.0	1600	3200	1600	500	800

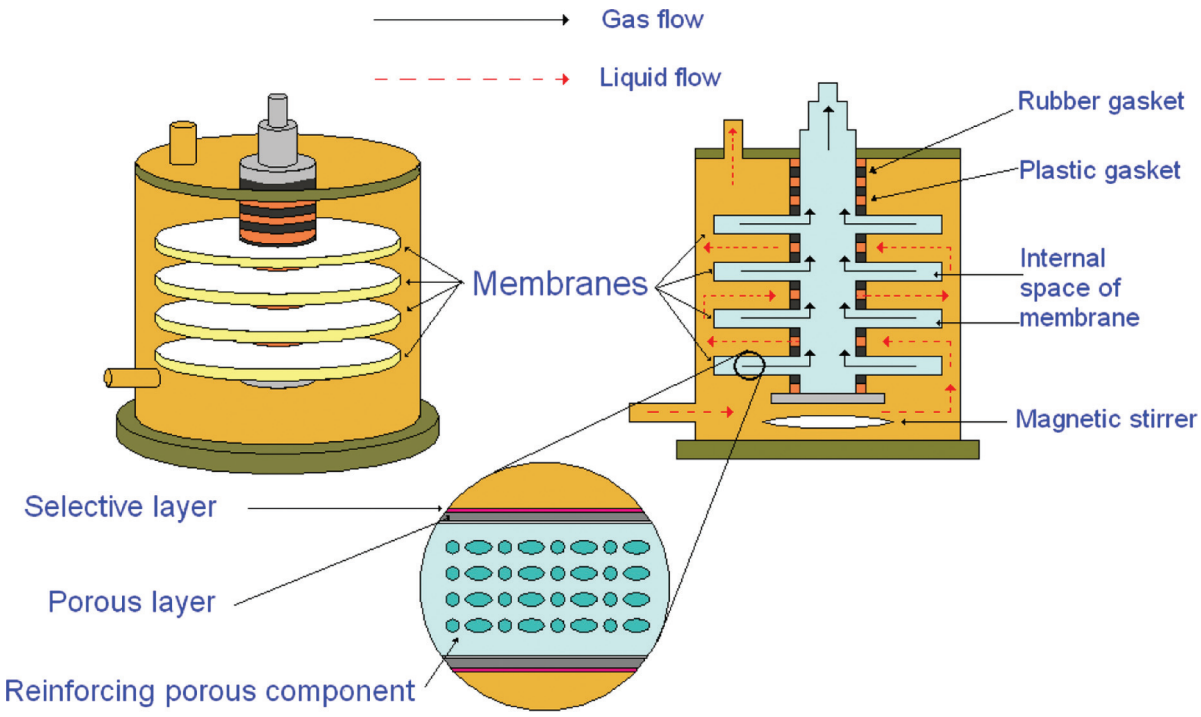

Fig. 3. Membrane module construction.

peristaltic pump (Fig. 5, 3). Gas mixture, contained mostly H_2 and CO_2, was removed from the liquid broth by vacuuming of membrane cartridges by vacuum pump (Fig. 5, 4).

Fermentation of cellulose by thermophilic microbial consortia in batch mode produces about 20 mM H_2 L^{-1} h^{-1}, whereas fermenter, integrated with membrane module, increased productivity up to 68 mM H_2 L^{-1} h^{-1}. This increase of production rate was obtained due to the constant removing of hydrogen from the liquid phase that prevent inhibition of cells productivity at high concentration of hydrogen.

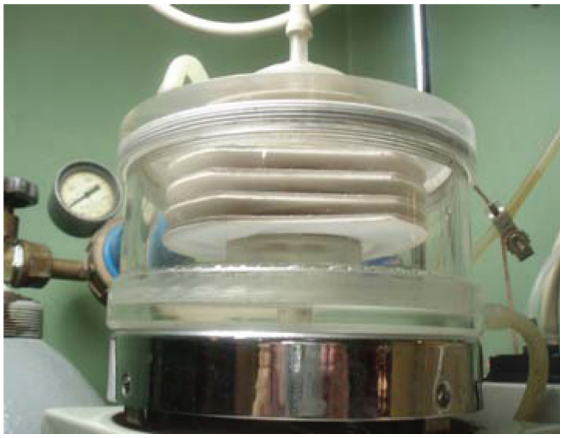

Fig. 4. The frontal view of membrane module mounted into plastic container.

3.2. Screening of the cellulose-degrading microbial consortia

Various sources of inoculation material were used for the selection of thermophilic cellulose-degrading microbial consortia. Samples were collected from herbivorous animal wastes of Moscow Zoo (elephant, zebra, black antelope, goat, giraffe, pony, cows etc.) as well as from various land sources where the natural decomposition of cellulose takes place: compost piles, decaying leafs, decomposing corn and wheat straw. Samples were anaerobically inoculated in two media in parallel (media 1 and 2, see Methods) and incubated at 60°C and 70°C. Enrichment cultures were subsequently sterile re-inoculated into the fresh media after visible decompositions of cellulose were observed. The pH was controlled (pH 6.5–7.5) by titration with sterile 0.1 M NaOH daily in all batch-flasks with enrichment cultures. Hydrogen production of enrichment cultures grown on cellulose as the sole carbon and energy source in media was monitored daily by measuring the pressure in the vessel and volumetric hydrogen content in the gas-phase.

Fig. 5. Laboratory set of equipment for continuous thermophilic cellulose fermentation integrated with membrane module. 1 – anaerobic reactor, 2 – membrane module, 3 – peristaltic pump, 4 – vacuum pump, 5 – PC with program control of overall fermentation process.

The most effective hydrogen producers were selected during the cellulose-degrading process (Fig. 6a, b). Comparative figures of growth in the media with cellulose and cellobiose (product of cellulose hydrolysis) showed that various enrichment cultures produce different amounts of hydrogen from two substrates. Enrichment culture #9 produced equal amount of hydrogen on cellulose and cellobiose whereas other cultures produced more hydrogen on cellobiose as the more easily fermented substrate compared with cellulose.

Maximal level of hydrogen productivity was recorded for the cultures #4 and #9. These cultures showed maximal yield of hydrogen at two temperatures (60°C, 70°C) on different substrates (cellulose or cellobiose).

The increased production of hydrogen on cellobiose (a direct product of cellulolytic activities of bacterial cellulases) can be explained by heterogeneous population of enrichment cultures where non-cellulolytic microbes can degrade cellobiose as well as cellulolytics. This can increase the net production of hydrogen by fermentative microbes compared to cellulose-containing medium where only cellulolytics can produce hydrogen from decomposition of cellulose.

The activities of enrichment cultures toward ability to natural and artificial wastes biodegradation were also compared. For this purpose we investigated hydrogen production of various enrichment cultures in the medium with sawdust compared to filter paper. Sawdust has (besides cellulose) certain amount of hemicelluloses, lignin and volatile oils. The last one can inhibit the activity of microbial consortia. Therefore, we tested the hydrogen productivity on various sawdust concentrations and it was shown that increasing the concentration of sawdust in the medium did not affect hydrogen production (Fig. 6c). The productivity was the same at 15 and 10 g/L substrate concentrations. Therefore the volatile oils did not reduce hydrogen production by enrichment culture #4. In contrast, increasing concentration of cellulose in the medium (filter paper) increased hydrogen production by enrichment culture #4 (Fig. 6c).

Another experiment was performed with newspaper wastes as the source of cellulose in the media. Enrichment cultures #4 was selected for this

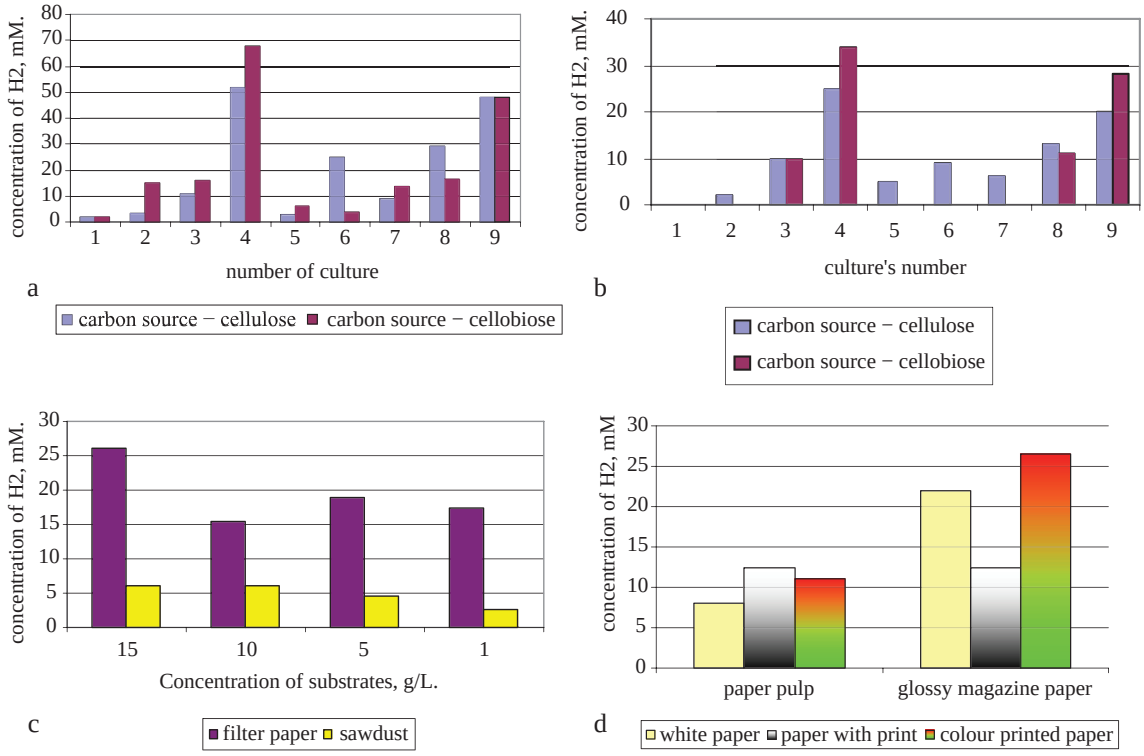

Fig. 6. Cellulose-degradation in different microbial consortia and biodegradation of different paper wastes. a – hydrogen production by various cellulose-degrading consortia upon growth on cellulose-containing media (filter paper) and cellobiose-containing media at 60°C; b – same as ''a'', but at 70°C (1 – anaerobe silt; 2 – grape presscake; 3 – bottom mud deposits of freshwater pond; 4 – chime of cows intestines; 5 – bottom mud deposits of waterlogged pond; 6 – chime of antelope intestines; 7 – chime of giraffe intestines; 8 – chime of pony intestines; 9 – wet tree waste); c – hydrogen production by selected consortium #4 on different types of substrates (cellulose and sawdust) at various concentrations at 60°C in 168 h; d – hydrogen production by selected consortium #4 on different types of waste paper at 60°C in 168 h.

experiment as the most productive one on cellulose at 60°C and 70°C. It shows high production of hydrogen from paper pulp as well as from glossy print paper of magazines. Color printed paper seems to be the most productive for hydrogen, possibly due to the containing of some amino acids (lysine) used for polishing of magazine print pages. Another explanation of increased hydrogen productivity on color print paper can be attributed to use of whitewash in the process of paper production. This chemical can increase the buffer capacity of the medium and therefore could lead to further increase of hydrogen production by the consortium.

Production of hydrogen on cellulose has limitation depending on hydrogen gas accumulation in the gas phase of the fermenter vessel. Therefore, the use of membrane-assisted device for instant hydrogen removal from fermentation broth can increase the production rate of hydrogen and lead to the deep cellulose conversion in the substrate.

4. Conclusion

Cellulose is a most abundant polymer containing glucose which produced on the Earth annually. Therefore, the conversion of this cheap, renewable and available substrate into the fuel gases can be very prospective source of renewable energy for the nearest future. Cellulose hydrolysis by aerobic brown-rot fungi is well-known process and cellulases of the fungi are available even for industrial processes of cellulose hydrolysis [13]. The anaerobic cellulose conversion into renewable fuels is more complex. Mostly known anaerobic bacteria are from *Clostridium* genus which can degrade cellulose anaerobically with producing of hydrogen and short-chain organic acids. This process is environmentally safe and can produce valuable fuel gases as well as organic liquids which can serve as the cheap chemical sources for the industrial purposes (acetic, butyric and lactic acids, ethanol, butanol and iso-propanol, etc.). In all these fermentation processes

evolving hydrogen can be used as renewable fuel for fuel cells as well as for direct combustion in various engines. Therefore, the production of hydrogen from the cellulose which is a renewable and available source of carbohydrates is an attractive process for the bioenergy obtaining.

Utilization of non-porous membranes for hydrogen recovery from fermentation broth increase the overall productivity of the microbial communities which can degrade cellulose anaerobically at elevated temperatures. This work demonstrates that submerged PVTMS membranes used in membrane fermentor can increase the productivity of microbial consortium degrading cellulose at 70°C up to 3 times (from 20 mM to 68 mM L^{-1} h^{-1}). This value is comparable with the most productive systems with cellulose substrate [17] reported in the world (12–15 mM L^{-1} h^{-1}).

PVTMS membranes applied to this study shown a good performance at elevated temperatures and good stability toward bifouling. This type of non-porous glassy-polymer membranes can be applied in the further work on hydrogen extraction from the fermentation broth in other processes involving biodegradation of cellulose with hydrogen production. The suitability and stability of PVTMS membranes for gas-phase separation, for hydrogen and methane removal from biological processes was already demonstrated [11,13]. This work shows the possibility of utilization of these membranes for direct hydrogen recovery from fermentation broth.

Acknowledgements

Authors thank FP6 Integrated Project SES-6 no. 019825 ''Non-thermal hydrogen production from biomass (''HYVOLUTION'')'' for support in part of this study.

References

[1] V.A. Vavilin and I. Angelidaki, Anaerobic degradation of solid material: importance of initiation centers for methanogenesis, mixing intensity, and 2D distributed model, Biotechnol. Bioeng., 89 (2005) 113–22.

[2] K. Lee, J. Wu, Y. Lo, Y.-C. Lo, P. Lin and J. Chang, Anaerobic hydrogen production with an efficient carrier-induced granular sludge bed bioreactor, Biotechnol. Bioeng., 87 (2004) 129–138.

[3] W. Park, S. Hyun, S. Eunoh, B. Logan and I. Kim, Removal of headspace CO_2 increases biological hydrogen production, Environ. Sci. Technol., 39 (2005) 412–420.

[4] D.-H. Kim, S.-K. Han, S.-H. Kim, H.S. Shin, Effect of gas sparging on continuous fermentative hydrogen production, Int. J. Hydrogen Energ., 31 (2006) 2158–2169.

[5] O. Mizuno, R. Dinsdale, F.R. Hawkes, D.L. Hawkes and T. Noike, Enhancement of hydrogen production from glucose by nitrogen gas sparging, Biores. Technol., 73 (2000) 59–65.

[6] J.-J. Lay, Modeling and optimization of anaerobic digested sludge converting starch to hydrogen, Biotechnol. Bioeng., 68 (2000) 269–278.

[7] I. Hussy, F.R. Hawkes, R. Dinsdale and D.L. Hawkes, Continuous fermentative hydrogen production from a wheat starch co-product by mixed microflora, Biotechnol. Bioeng., 84 (2003) 619–626.

[8] M. Cooney, N. Maynard, C. Cannizzaro, D. Harris, On-line measurement of gas production rates, Biotechnol. Prog., 22 (2006) 1456–1460.

[9] H. Hammon, K. Ernst, R. Newton, Noble gas permeability of polymer films and coatings, J. Appl. Polym. Sci., 21 (1977) 1989–1997.

[10] P.S. Kumar, J.A. Hogendoorn, P.H.M. Feron, G.F. Versten, Approximate solution to predict the enhancement factor for the reactive absorption of a gas in a liquid flowing through a microporous membrane hollow fiber, J. Membr. Sci., 231 (2003) 231–245.

[11] L.G. Gasanova, A.I. Netrusov, V.V. Teplyakov and M. Modigell, Fuel gases from organic waste by using membrane bioreactors, Desalination, 198 (2006) 56–66.

[12] A.P. Korikov and K.K. Sirkar, Membrane gas permeance in gas-liquid membrane contactor systems for solutions containing a highly reactive absorbent, J. Membr. Sci., 246 (2005) 27–37.

[13] L. Gasanova, T. Mitrofanova, N. Zotova, V. Teplyakov, A. Netrusov and M. Modigell, Separation of fuel gases of microbial origin by using active membrane systems, Desalination, 200 (2006) 588–590.

[14] V.V. Teplyakov, L.G. Gasanova, E.G. Sostina, E.V. Slepova, M. Modigell and A.I. Netrusov, Lab-scale bioreactor integrated with active membrane system for hydrogen production: experience and prospects, Int. J. Hydrogen Energy, 27 (2002) 1149–1155.

[15] A.A. Imshenetskij, Microbiology of cellulose, Moscow (USSR), Nauka Publishing House, 222 (1953) (in Russian).

[16] J.W. van Groenestijn, J.S. Geelhoed, H.P. Goorissen, K.P. Meesters, A.J. Stams and P.A. Claassen, Performance and population analysis of a non-sterile trickle bed reactor inoculated with Caldicellulosiruptor saccharolyticus, a thermophilic hydrogen producer, Biotechnol. Bioeng., 102 (2009) 1361–1367.

[17] T. de Vrije, R.R.Bakker, M.A.W.Budde, M.H.Lai, A.M.Mars, P.A.M.Claassen, Efficient hydrogen production from the lignocellulosic energy crop Miscanthus by the extreme thermophilic bacteria *Caldicellulosiruptor saccharolyticus* and *Thermotoga neapolitana*, Biotechnol. Biofuel., 2 (2009) 12–21.