Hungarian Association of Agricultural Informatics
European Federation for Information Technology in Agriculture, Food and the Environment

Journal of Agricultural Informatics. 2014 Vol. 5, No. 2

Process simulation of integrated biohydrogen production: hydrogen recovery by membrane separation

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I N F O
Received 22 Sept 2014
Accepted 29 Sept 2014
Available on-line 6 Oct 2014
Responsible Editor: K. Rajkai

Keywords:
biohydrogen, *Escherichia coli*, process simulation, continuous product removal, membrane gas separation

A B S T R A C T

In this project, the production of biohydrogen, as a renewable and sustainable energy source was studied. Biohydrogen was manufactured by using *E. coli* strain in a batch dark fermentative process integrated with membrane gas separation. Two different methods were applied: Firstly, the amount of the produced gas and component concentrations were measured, but CO_2 and H_2 gases were not separated. In the second experiment CO_2 was removed from the gas mixture via chemical sorption (reacting with NaOH). Both methods use continuous product removal in order to enhance the biohydrogen formation. In addition, process modeling was carried out with a simulation software (SuperPro Designer, Intelligen Inc.) so that experimental and computational results could be compared. CO_2 and H_2 flow rates and fluxes were calculated on the basis of the membrane permeation data obtained by using pure gases and silicone (PDMS) hollow-fiber membrane module (PermSelect – MedArray Inc.).

1. Introduction

Currently the importance of the agriculture connected renewable and sustainable energy is higher and higher. The amounts of wastes coming from agriculture are tremendous; their usage for e.g. energy can be a key factor in future. Furthermore the demand for energy is steadily increasing in industry and also in everyday life; therefore intensive research is still necessary in order to resolve the problem. The main goal is to find and develop suitable methods for high-efficiency and economical production and utilization of second generation energy sources (e.g. agricultural wastes).

Hydrogen can be highly beneficial in several points of view because it has a high enthalpy of combustion and furthermore is an environmental-friendly fuel. The main raw materials from which hydrogen can be produced are natural gas, coal, methanol, ethanol, gasoline, methane, biomass and

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water [1]. Classical techniques to generate hydrogen apply generally fossil fuels – particularly steam reforming of natural gas - and need a lot of energy [2]. Because of the increasing costs and environmental impact the biological hydrogen production catalyzed by microorganisms is a promising solution [3]. Biohydrogen is one of the most emerging renewable energy carriers in addition to the more eco-friendly technology such as wind, hydropower, biofuel, solar and geothermal energy, etc. There are two main possibilities for hydrogen formation via biological conversion pathway: light-dependent processes and dark fermentation [4].

In the course of photosynthetic systems the photo synthetically active pigments absorb light, then – in bio-photolysis, occurs in cyanobacteria and green algae strains - water is split by the energy derived from the photon absorption; in addition, O_2 and H_2 are evolved (in case of green plants CO_2 reduction takes place due to the absence of hydrogenase) [5,6]. However, forming O_2 is the inhibitor of the reaction and O_2-H_2 mixture is explosive [7]. In case of photofermentative microorganisms – mainly purple non-sulfur bacteria and green sulfur bacteria such as *Rhodobacter spheroids* and *Chlorobium vibrioforme* – which can produce H_2 by consuming short-chain organic acids [8,9]. The advance of the process depends on the presence of ATP, which is necessary for the enzymatic reaction catalyzed by nitrogenase [10]. Both methods show low efficiency and high sensitivity [11].

Currently, dark fermentation is apparently the most viable way for hydrogen production [12]. Carbohydrate-rich substrates, e.g. cellulose and starch, are converted by anaerobic bacteria into biohydrogen and other organic substances without light [13]. The forming gas contains H_2, CO_2, and also less amounts of N_2 and trace gases e.g. H_2S, however, PEM fuel cells which can convert hydrogen into electricity with significant efficiency, require purified H_2 [13]. Therefore, gas separation techniques become more and more important and fermentative processes integrated with membrane gas separation are of particular research interest. Such combined systems have several advantages, including enhancement of hydrogen formation if the product (H_2) is continuously removed. Besides, purification begins already during fermentation, thus helps the downstream processes.

In addition to more microbial species, facultative anaerobic *Escherichia coli* bacteria can be effective for hydrogen production, since it is fast-growing and also well-studied in biotechnological operations [14]. This strain prefers mesophilic circumstances and is able to oxidize hydrogen. H_2 and CO_2 formation through mixed-acid fermentation from formate is catalyzed by formate hydrogenlyase (FHL) enzymatic complex [15,16]. The different kinds of glucose catabolism in *E. coli* are shown in *Figure 1*. Glucose is considered as a result of various agricultural wastes, since it is formed by degradation of cellulose and starch.

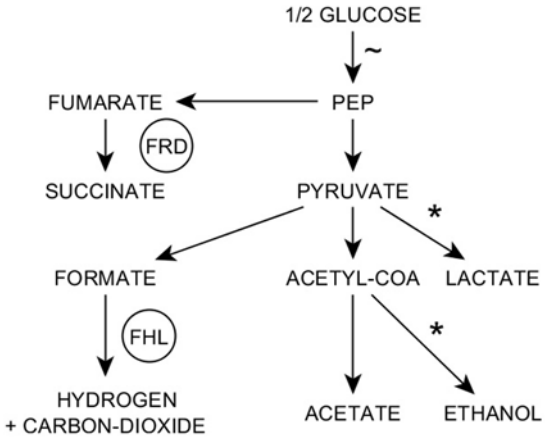

Figure 1. Metabolic pathways of H_2 formation in *E. coli* from glucose substrate. H_2 and CO_2 are formed in equimolar amounts. Abbreviations: PEP, phosphoenol-pyruvate; FRD, fumarate reductase; FHL, formate hydrogenlyase complex. Simbols: ~, $NAD^+ \rightarrow NADH$ reaction; *, $NADH \rightarrow NAD^+$ reaction

Computational methods can be also used in biotechnology for the comparative study of system behaviour. As a novelty, modelling of fermentation was set up with simulation software by using

defined initial parameters such as temperature, amount of substrate, reaction kinetics and considered approximations of the model. In this case, process modelling allows the rapid examination of membrane separation efficiency and of residence time influence, thus may facilitate the design.

2. Materials and methods

2.1. Inoculum preparation

In this work *E. coli* (XL1-BLUE) strain and LB broth that contained tryptone (10 g/l), yeast extract (5 g/l) and NaCl (5 g/l) were used. The broth was sterilized and then inoculated with *E. coli*. The culture was grown for 24 h (at 37 °C, agitation rate of 150 rpm).

2.2. Fermentation setup and experimental design

The first fermentation method was performed as a baseline where the formed gases were not separated. The Biostat B plus Sartorius Stedim glass-made tank reactor, with a working volume of 2 l, contained tryptone (10 g/l), yeast extract (5 g/l), NaCl (3.33 g/l) as fermentation media, and added sodium-formate (2.04 g/l) as the limiting substrate of the reaction. Openings were fitted with sterile filters and hose-pipes were clamped. After sterilization the fermenter was allowed to cool down prior to inoculation. NaOH and H_2SO_4 buffers (20 w/w%) were used to keep the desired pH level. After flushing with high-purity nitrogen, the fermentation and the collection of data were started with the following parameters: stirring rate – 220 rpm, temperature – 37 °C and pH – 6.5.

The second experiment was carried out with the following change: CO_2 was removed via chemical sorption. For this purpose the reaction with NaOH was chosen, 4 l/h off-gas from the bioreactor was passed through a tank containing NaOH, then it was fed back to the bottom of the reactor. Thus, the concentration of CO_2 was reduced to zero.

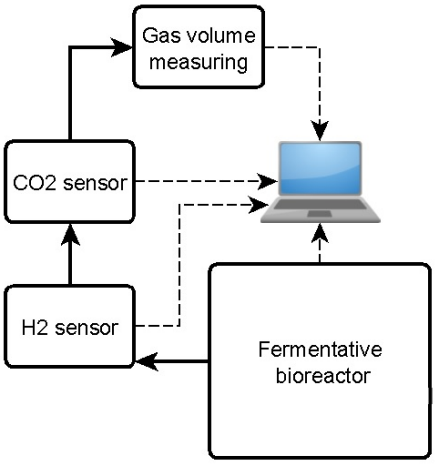

Figure 2. Experimental hydrogen fermentation

2.3. Analysis

The concentration and pressure of H_2 and CO_2 were measured by using BlueSens gas analyzer and related software. The data were recorded by sampling every 10 minutes. To determine the volume of generated gases, air lock system was used and the received signals were processed with Velleman software. The volume of produced hydrogen was calculated as described by Bakonyi et al [20].

2.4. Membrane gas separation

Gas separation was carried out with silicone (PDMS, polydimethylsiloxane) hollow-fiber membrane module (PermSelect - MedArray Inc.). The module contained 30 pieces of non-porous silicone fibers in a bundle. Pressure and temperature limits of the PDMS module are 3 bar and 60 °C. Permeation characteristics were measured at different pressures by using pure H_2 and CO_2 gases and

bubble flow meter. The system was thermo stated to 15 °C. The flux (□) of gases – thus the theoretical selectivity – was calculated on the basis of volumetric permeation rates. The number of the required membrane modules for the suitable separation can be estimated from these data.

2.5. Simulation design

Simulations were carried out by using SuperPro Designer software (Intelligen Inc.) applying different initial conditions. Biomass (3.5%), glucose (0.5%) and water (96%) were fed with a total mass of 109.86 kg as raw materials to the reactor. The headspace of the bioreactor was flushed with N_2 (0.1 mol). Fermentation temperature was adjusted to 37 °C. Two reactions took place inside, which were adopted from SuperPro Designer (*Eq.(1)* and *Eq.(2)*).

$$(1) \quad 180g\ C_6H_{12}O_6 + 108g\ H_2O \rightarrow 264g\ CO_2 + 24g\ H_2$$

$$(2) \quad 180g\ C_6H_{12}O_6 + 73.52g\ biomass + 106.48g\ CO_2$$

Both reactions were limited by glucose. The system was simplified by introducing the following approximations:

(i) The formed gases are insoluble in water

(ii) Water is non-evaporable

(iii) Materials are not left in the unit operations

The fermentation kinetics was set based on the Monod-model formalized in *Eq.(3)*.

$$(3) \quad \mu = \mu_{\max} \frac{S}{K_S + S},$$

where μ is the specific growth rate, S is the concentration of the limiting substrate for growth, K_s is the half saturation constant. These values can be seen in *Table 1*.

Table 1. Values of the Monod equation parameters for each reaction

Reaction	μ_{max} (l/h)	K_s (mg/l)
(1)	0.05	35
(2)	0.39	100

Under these conditions, simulations were run applying different hydraulic residence times (5 h, 7 h and 9 h). Firstly, the compiled fermentation system (FS1) contained three unit operations (*Figure 3*): bioreactor (P-4/V-102) and 2 filter module (P-5/BHF-103, P-6/BHF-104). To improve the separation efficiency, the model was supplemented by a mixer (P-7/MX-101) and the hydrogen-rich gas stream (S-103) was recirculated and mixed with the reactor leaving gas (S-108) as it can be seen in *Figure 4* (FS2).

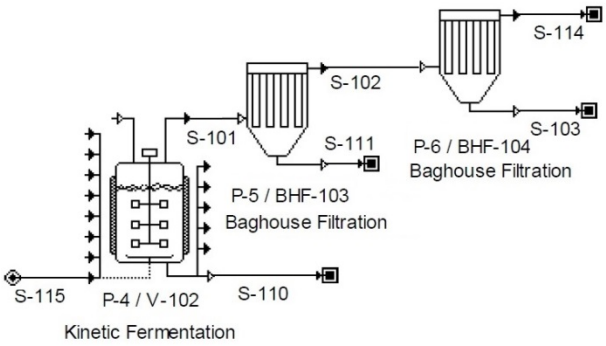

Figure 3. Flow chart of simulated hydrogen fermentation without recirculation (FS1)

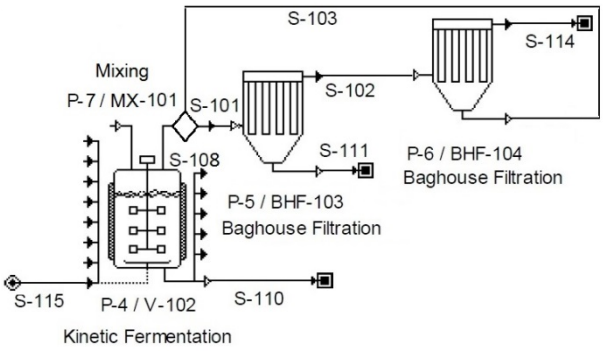

Figure 4. Flow chart of simulated hydrogen fermentation with the recirculation of hydrogen-rich gas stream (FS2)

The concept of the product yield was introduced for the characterization of the membrane gas separation efficiency and it was formalized as follows:

$$\eta = \frac{n}{N} \cdot 100\,\%, \quad (4)$$

where n is the amount of H_2 in the purified gas stream and N is the amount of all the produced H_2.

3. Results and discussion

3.1. *Results of fermentation measurements*

In this work the assumption that the gas production of the cells can be enhanced by continuous product removal was examined. Therefore, the pressure in the reactor had to be constant during the experiments (in our case 1 bar). To verify it, the pressure was measured, and the data showed that the systems were not leaked.

The first fermentation was lasted for 48 hours. The volume of all the produced gas was almost 1000 ml and it has monotonously increased as function of time. There was not significant change in the hydrogen concentration during the first five hours, it was less than 1 Vol.%. In the following 40 hours this value exceeded 50 Vol.%. The changes of the hydrogen concentration and of the H_2 production rate are shown in *Figure 5* as a function of time.

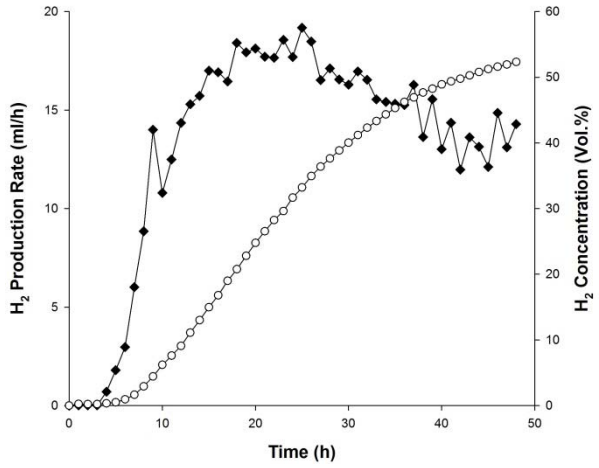

Figure 5. The time course of the hydrogen production without CO_2 separation. Black diamond – H_2 production rate; white circle – H_2 concentration

The H_2 production rate of *E. coli* reached its maximum around 20 ml/h after 18 hours. After the 25th hour the amount of evolving hydrogen was decreased 0.5 ml per hour, as it can be seen in hydrogen production rate curve. Summarizing these values, all the produced hydrogen was 640 ml.

The linear increase could also be observed in the second fermentation combined with CO_2 removal. The volume of the produced gas was 740 ml during the 36-hour process, which contained 683 ml of hydrogen. The H_2 production rate was higher than 20 ml/h from the second hour, increased up to 30 - 35 ml/h, and the concentration of H_2 exceeded 50 Vol.% already after 20 hours (*Figure 6*). The amount of evolved H_2 began to decrease from the sixth hour with a rate of 0.75 ml/h.

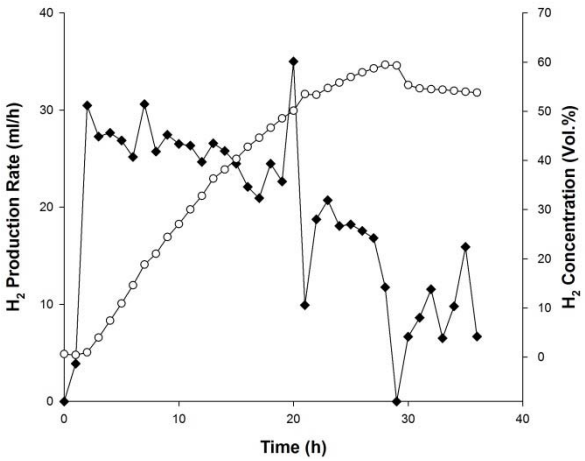

Figure 6. The time course of the hydrogen production with CO_2 removal. Black diamond – H_2 production rate; white circle – H_2 concentration

CO_2 and O_2 concentrations were determined in order to verify the separation level and the adequacy of the anaerobic conditions. The data showed that passing the obtained biological gas mixture through NaOH solution could ensure quite low residual CO_2 content in the purified gas stream as low as ~2 Vol.% that is, however, should be taken into account during the consequent membrane separation step. After flushing with nitrogen, oxygen was displaced; its concentration was below 1 Vol.% on average. Thus, we could provide the anaerobic circumstances during the process. The total hydrogen productions of the fermentations are compared in *Figure 7*.

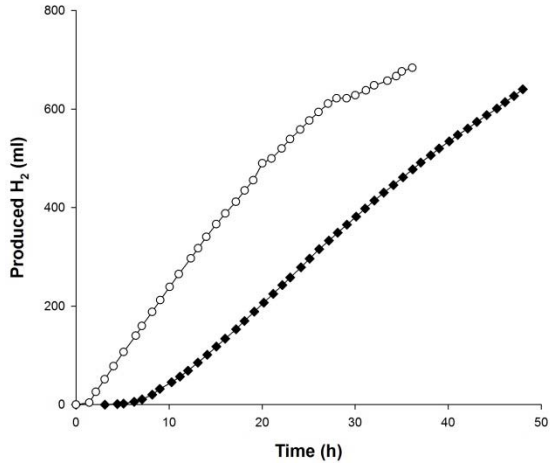

Figure 7. The comparison of the hydrogen production in two fermentations. Black diamond – fermentation without CO_2 removal; white circle – fermentation with CO_2 removal

It can be concluded that the separation resulted in higher hydrogen production rate and also in a shorter lag phase time in case of in-situ CO_2 removal. However, similar final amounts of H_2 were generated. Overall, 600 ml of hydrogen production was achieved 18 hours faster by using CO_2 separation, which may be advantageous in case of repeated fermentations.

3.2. *Results of process simulations*

Fermentations were supplemented with simulation data. Thereby the required parameters for the optimal operation of the real system can be estimated.

Calculations with FS1 confirmed the observation that the amount of the produced hydrogen and the consumed glucose increased as a linear function of residence time (*Figure 8*). The recirculation was carried out by external mixing – unlike the experiment where the gas was fed back to the bottom of the reactor –, thus it had no effect on the fermentation. Therefore no significant difference in values was observed in the case of the FS2 system. The results showed that the highest reachable hydrogen concentration was slightly more than 72 mol%.

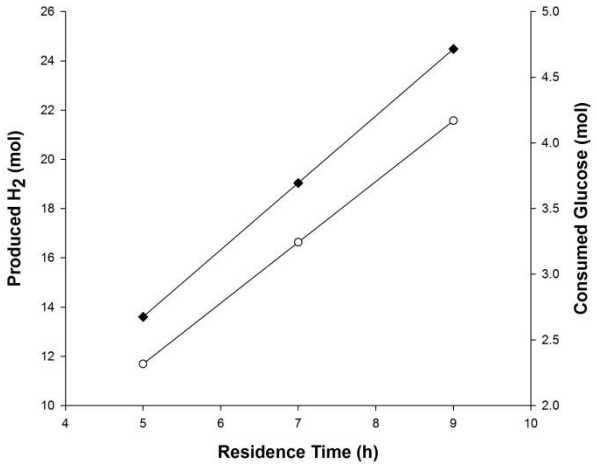

Figure 8. The obtained amount of substances from the simulations. Black diamond – produced H_2; white circle – consumed glucose

The gas feedback resulted in an increase of H_2 amount in the purified stream as compared to total H_2 production.

Table 2. The amount of produced and purified H_2 in the fermentation systems

Residence time (h)	Produced H_2 (mol)	Purified H_2 in FS1 (mol)	Purified H_2 in FS2 (mol)
5	13.6	8.8	11.4
7	19	12.4	16
9	24.5	15.9	20.6

As it can be seen in *Table 2*, the product yield in the FS1 was not more than η=65%, however, it could positively be influenced by the recirculation of the hydrogen-rich gas stream. The separation efficiency was better in the FS2 and η=84% could be reached. It was a further advantage that there was only one output which contained hydrogen after leaving the second membrane module, thus the loss was barely more than 15% even if it was not utilized, while this value was 35% in the FS1.

3.3. *Membrane gas separation for hydrogen purification*

Single gas experiments were carried out at different pressures in order to determine volumetric rates of CO_2 and H_2. The retentate stream was closed during the permeation time measurements.

Faster permeation of CO_2 through the membrane was observed (*Figure 9*). Theoretical CO_2/H_2 selectivity (α) was calculated on the basis of the fitted lines (*Table 3*).

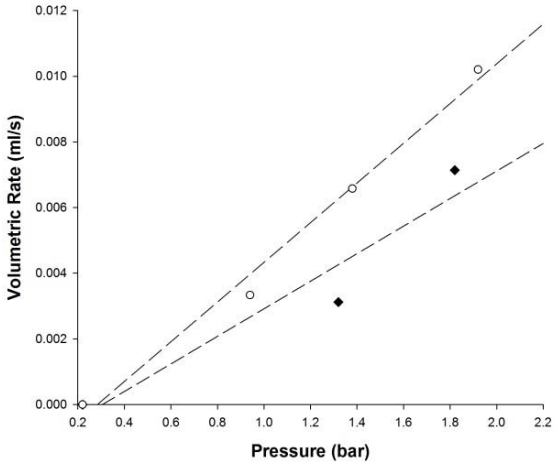

Figure 9. Volumetric rates of pure gases as a function of pressure. Black diamond – H_2; white circle – CO_2

Table 3. Flux of gases (ν) and theoretical CO_2/H_2 selectivity (α) at different pressures

Pressure (bar)	ν_{CO2} ($lh^{-1}m^{-2}$)	ν_{H2} ($lh^{-1}m^{-2}$)	α
1	15.48	10.44	1.48
2	37.08	25.56	1.45
3	58.68	40.68	1.44

The results showed in Table 3 that the PermSelect membrane was suitable for the separation and higher theoretical selectivity was attainable at lower pressures. Most of the CO_2 was separated in the permeate stream, therefore H_2 could be recovered in the retentate stream because of the CO_2 selectivity of PDMS.

Based on the results with the unusual, H_2 selective membranes we concluded that not only the CO_2 selective membranes are suitable to construct integrated systems. Applying proper conditions, these membranes could be used to build fermentation – gas separation combined systems, resulting in higher effectiveness.

4. Conclusions

In this work biohydrogen fermentation by *E. coli* and membrane separation of the formed gas were studied via two different methods. Continuous product removal was used during both fermentative processes in order to enhance hydrogen formation. The results were supplemented with computational data derived from process simulations, where the residence time influence on H_2 production and the separation properties were examined. It can be concluded, that the product and the sorption CO_2 removal resulted in faster H_2 formation. Simulations showed monotonous increase in the amount of hydrogen as a function of time, as it was also observed in the experiments. The introduction of the recirculation caused 19% higher product yield. It was found that membrane separation can be useful for the purification of biohydrogen with the tested PermSelect membrane module.

Acknowledgement

Péter Bakonyi thanks for the TÁMOP 4.2.4.A/2-11-1-2012-0001 'National Excellence Program' supported by the European Union and the State of Hungary, co-financed by the European Social Fund. Nándor Nemestóthy acknowledges the János Bolyai Research Scholarship of the Hungarian Academy of Sciences. The research work was partly supported by the „Social Renewal Operational Programme" (New Széchenyi Plan) in the frame of TÁMOP-4.2.2.A-11/1/KONV-2012-0038 project, entitled „Complex multidisciplinary investigation of the effects of human activity and the related social conflicts on a sensitive geographical area pertaining to a shallow lake (the water body of Lake Balaton and its southern catchment area). The project is supported by the European Union and co-financed by the European social Fund.

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