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Biohydrogen production in integrated system

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ABSTRACT

Biohydrogen formation during the fermentation carried out by an *Escherichia coli* strain was studied and its recovery, purification was investigated by a polymeric gas separation module. The aim is to design and build an integrated system, where a membrane module is connected to the fermenter and purified biohydrogen obtained is to use directly in a PEM fuel cell.

Keywords: *Escherichia coli*; Gas separation; Sodium formate

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. There are two main possibilities for biohydrogen formation: photosynthetic way and dark fermentation. In this research work dark fermentation by *Escherichia coli* strain was studied. *E. coli* is a bacteria, widely used in experimental molecular biology. Under anaerobic conditions it is able to produce hydrogen by its different hydrogenase enzymes [1–4]. The bacteria has three active and an inactive hydrogenase enzymes, among the active ones hydrogenase I and II act in the hydrogen consumption process [5], while hydrogenase II forms hydrogen within the cell. Moreover formate hydrogenase lyase enzyme is also able to synthesize hydrogen in the presence of formate in conjunction with hydrogenase III and formate dehydrogenase enzyme [6]. In order to enhance hydrogen production this complex biochemical system should be controlled properly.

During biohydrogen fermentation a gaseous mixture is present in the headspace of the fermenter. It contains nitrogen (to maintain initially the anaerobic atmosphere), water vapour from the aqueous broth, and more and more hydrogen and carbon dioxide formed in the fermentation process. The pressure increase in the fermenter indicates the gas formation in the system.

The gas mixture – containing hydrogen – obtained in the fermentation process, however, is not suitable for direct utilisation, therefore it should be separated, which can be carried out by membrane gas separation [7–9]. In biological systems usually polymeric gas separation membranes have been used, mainly for CO_2 separation [10–12].

2. Materials and methods

During the fermentation experiments *E. coli* XL1-BLUE strain (received from Szeged University, Hungary) was used. To maintain the strain LB broth (Sigma) was applied. Luria-Bertani broth, also known as LB broth is a commonly used liquid medium to grow bacteria such as *E. coli*, it consists of three main ingredients: yeast extract, tryptone, and sodium chloride (salt). Fermentations were carried out in a WTW OXITOP 100 type manometrical BOD equipment,

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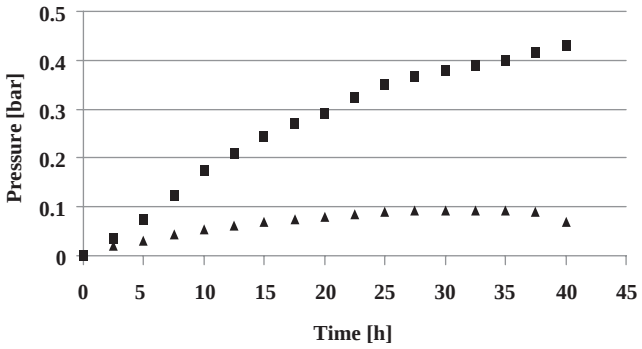

Fig. 1. The effect of formate presence on hydrogen formation (squares: LB + formate; triangles: LB only).

where the growing pressure – caused by the biohydrogen formation – was measured.

The composition of the gaseous mixture formed during the fermentation was determined by gas chromatography. To measure hydrogen content a GOW-MAC Series 600 type GC was used, with helium as a make-up gas, 2 m long zeolite (X13) packed column and thermal conductivity detector (TCD) detector. For CO_2 determination HP 5890 series II GC was applied, with nitrogen gas, Carboplot column and TCD detector.

In the gas separation measurements PES-PI non-porous hollow fiber membrane built in a high pressure, stainless steel module was used. The effective membrane surface area was 9 cm^2, the length of the fibres was 15 cm. The feed gaseous mixture was introduced from the shell side of the modules at 2–2.5 bar pressure from a buffer vessel (2.4 l volume), while permeate was obtained from the inner side of the fibres. To test the membrane module single gases were used in the first serial of measurements, determining the permeation rates at 40°C temperature.

3. Results

3.1. Fermentation experiments

According to the recent literature data on the biochemical pathways, formate may have an important role in the hydrogen formation by *E. coli*. To find out the correlation experiments were carried out using LB broth with and without sodium formate supplement. In Fig. 1 the effect of formate addition is shown.

As it can be seen the amount of biohydrogen was significantly increased in the case of formate addition, hence further experiments were carried out using LB broth completed with formate.

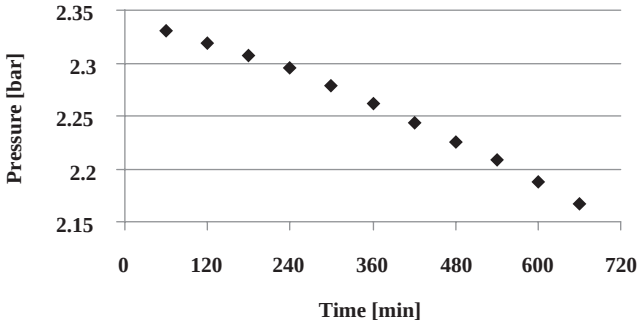

Fig. 2. Hydrogen permeation data.

Table 1
Permeation of single gases

	Hydrogen	Carbon dioxide	Nitrogen
Permeation [GPU]	16.1	3.55	0.76

3.2. Gas separation measurements

Gas separation measurements were carried out using single gases: hydrogen, nitrogen and carbon dioxide. The data of the decreasing pressure of the feed gas were followed and recorded as a function of time. As an example the measured data for hydrogen are presented in Fig. 2. It can be seen that pressure decreased rather quickly due to the high gas permeation through the membrane thus the measurements were conducted in a narrow range of pressure.

Based on the measurements with the single gases, permeations were calculated taken into account the membrane surface area of the module and summarized in Table 1.

The theoretical selectivity values were calculated as a ratio of the permeation rates for the various gases. These values are summarized in Table 2.

It was found – based on the experimental results – that the gas separation membrane has high selectivity and permeability. The H_2/N_2 selectivity is remarkably high, while the H_2/CO_2 selectivity is good enough for the CO_2 separation. Based on these values it can be concluded that the membrane module is suitable for the one-step separation of the gaseous mixture formed during biohydrogen fermentation.

4. Conclusions

In this work biohydrogen fermentation by *E. coli* and separation of the gas formed by membranes were

Table 2
The theoretical selectivities

	Hydrogen	Carbon dioxide	Nitrogen
Hydrogen	–	4.53	21.18
Carbon dioxide	0.22	–	4.67
Nitrogen	0.05	0.22	–

studied. It was found that formate has a significant effect on the biohydrogen production. For the separation of the gaseous mixture formed in the headspace of the fermentor a non-porous hollow fiber membrane module was tested. Based on its permeability data for single gases we found the membrane suitable for the one-step separation. In the near future experiments are planned where in an integrated system – coupling the fermenter and the membrane gas separation – purified biohydrogen will be produced, which may be used directly in fuel cells.

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