BlueSens

understanding bioprocesses

Application Report

Report from

Péter Bakonyi, Nándor Nemestóthy, Katalin Bélafi-Bakó

Research Institute on Bioengineering, Membrane Technology and Energetics
University of Pannonia
Egyetem ut 10, 8200 Veszprém, Hungary

Report on the application of BlueSens® gas sensor in continuous bioH_2 process optimization

Introduction

Hydrogen production technologies have received remarkable attention in the recent years due to the great increase in H_2 demand as a feedstock in different industries. Nevertheless, hydrogen is considered as a clean energy carrier expected to play an important role in future fuel cells for cars, portable electric devices, etc. It is an ideal and completely environmental-friend energy source due to its combustion resulting only water as product. Nowadays, the conventional hydrogen production methods mostly based on the steam reforming of fossil fuels e.g. natural gas or oil are operated at high temperature and pressure and disadvantageous from environmental point of view. In contrast, biological hydrogen production processes from cheap, renewable resources take place under nearly ambient circumstances and thus offer promising way to replace traditional methods without emitting any pollution to the atmosphere. The bioprocesses for hydrogen production can be classified into two main categories: the photosynthetic and the dark fermentation processes. Nowadays, compared to light-driven hydrogen bioproduction, anaerobic dark hydrogen fermentation is more feasible for practical application due to its higher efficiency, higher stability, simpler control requirements, etc. According to the predictions, petroleum economy may transit to hydrogen economy in the next few decades if obstacles including the lack of a reliable and sufficient supply of (bio)hydrogen can be overcome. Therefore, proper bioreactors need to be designed and built-up using efficient microbes. Recently, a large number of microorganisms and substrates have been used and investigated for biohydrogen formation and *Escherichia coli* has been found to be an attractive strain for bacterial hydrogen production. This strain can generate hydrogen using various substrates among which formate was shown to ensure the highest hydrogen productivity. Moreover, formic acid can be derived from low cost renewable materials, such as biomass. In our previous study, *E. coli* (XL1-BLUE) was proven to be able to produce biohydrogen in batch experiments under anerobic circumstances. In this work, the objective was to establish a continuous process for hydrogen bioproduction using the same

organism. To test the ability of *E. coli* cells to grow anaerobically and evolve hydrogen at the same time in a continuous system, a well-mixed continuous tank reactor was employed in this study. For a continuous process, hydraulic retention time (HRT) is known as an important operational factor which could significantly influence the performance of the biosystem. Therefore, its impact on hydrogen generation was focused. In the first series of experiments, kinetic study was performed in order to determine the exponential phase of bacterial growth and thus, predict the proper hydraulic retention time to be used in a continuous system. To our knowledge, the investigation of the suitable HRT for improved continuous biohydrogen fermentation using cell growth kinetic has not been applied hence, this was aimed now. Based on the data obtained from kinetic study, measurements were carried out in a continuously stirred tank reactor (CSTR) using a BlueSens real-time biohydrogen analyzer at various dilution rates in order to find the proper microbial residence time that should be used for continuous biohydrogen production. CSTR systems have been widely applied for fundamental studies of continuous hydrogen fermentation due to its homogenous and good mass transport properties.

Materials and Methods

Study on bacterial growth kinetic

To investigate the dependence of bacterial growth on the composition of nutrient broth *E. coli* (XL1-BLUE) cells were applied in a glass-made tank reactor with a total volume of 2 l (220 rpm stirring rate, 37 °C, pH 6.5) containing sterile fermentation media: tryptone 10 g l^{-1}, yeast extract 5 g l^{-1} and NaCl 3.33 g l^{-1}. The broth also contained formate as the limiting carbon source at various concentrations ranging between 1-5 g l^{-1}. The initial cell concentration in each cases was adjusted to 0.05 g dry cell weight (dcw) l^{-1}, the volume of liquid phase was 1000 ml, temperature and pH were maintained constant at 37 °C and 6.5, respectively. After inoculation, the fermenter was flushed with high-purity nitrogen for 20 minutes in order to ensure the anaerobic conditions. Afterwards, liquid samples were taken manually from the reactor in consecutive time periods and the cell density (*X*) was measured spectrophotometrically at 620 nm wavelenght (Fig. 2).

Biohydrogen fermentation in continuous system using a real-time BlueSens hydrogen analyzer

The bacterial cells were inoculated into a 2 l glass tank reactor containing sterile nutrient solution in which the levels of ingredients were set as found to be optimal with batch cultures in previuous study: formate 30 mM, tryptone, 10 g/l, yeast extract 5 g/l and NaCl 3.33 g/l. The anaerobic conditions were provided by nitrogen purging, the circumstances regarding the initial cell density, temperature and pH were the same as described for the kinetic study. The initial cell density for all cases was accurately adjusted to 0.05 g dcw l^{-1}. After 8 hours of start-up stage, when cell growth reached the initial stacionary phase, the reactor was switched to continuous mode. During this time, fresh nutrient solution – with the same composition as given for broth – was continuously fed into the reactor from an external vessel (working volume 4.5 l) and effluent was removed simultaneously at various dilution rates by a peristaltic pump ($D=FV^{-1}=HRT^{-1}$, where D is the dilution rate (h^{-1}), F is the flow rate of influent (ml h^{-1}), V is the total volume of liquid phase (ml) inside the reactor and HRT is the hydraulic retention time (h). During the experiments, the total volume of reaction volume (V) in the reactor was kept constant at 1000 ml. Each trials were run in continuous mode at least for approximately 4 cycles of the applied HRT (Table 1). The efficiency of continuous process operated under different hydraulic retention times was evaulated by calculating both hydrogen yields (mmol H_2 produced/mmol formate) and productivities (mmol H_2 $l^{-1}h^{-1}$). During the fermentations, a real-time hydrogen analyzer – product of BlueSens Gas Sensor GmbH (Germany) – was employed for monitoring the concentration of hydrogen formed due to the microbial activity. The output signal of the sensor displays the percentage volume of H_2 in the headspace of the

bioreactor. The device has a measuring range of 0-100% H_2 with a minimal response time of 20 s in the temperature range of 25-55 °C. In our case, the measuring frequency of hydrogen content was set to 1 h^{-1}. Benefits of applying the BlueSens hydrogen sensor is that the on-line gas analysis can provide a significantly improved process monitoring and control compared to the traditional off-line gas composition analyzis performed by gas chromatography where frequent calibrations, expensieve carrier gases and manual sampling need to be used. Gas production was measured by a volumetric gas meter based on the water displacement method. The amount of hydrogen formed was calculated using the following equation (Eq. 1):

$$V_{H,i} = V_0(C_{H,i} - C_{H,i-1}) + C_{H,i}(V_{G,i} - V_{G,i-1})$$

where $V_{H,i}$ is the volume of biohydrogen produced at (i) time interval, V_0 is the volume of the headspace, $C_{H,i}$ and $C_{H,i-1}$ are the volume fractions of biohydrogen gas at the current (i) and the previous (i-1) time intervals in the headspace of the fermenter, respectively while $V_{G,i}$ and $V_{G,i-1}$ are the total volumes of gas produced at the current (i) and the previous (i-1) time intervals, respectively.

The experimental biorector set-up is shown in Fig. 1.

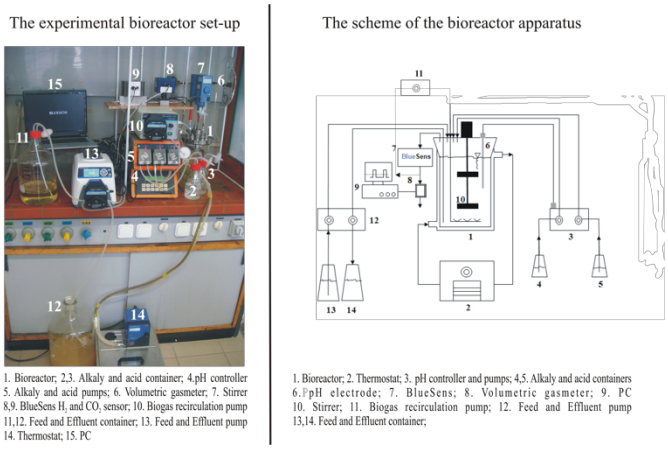


Fig. 1 – The experimental hydrogen producing biosystem

Results and Discussion

The determination of exponential growth phase and specific growth rates

As described above, *E. coli* (XL1-BLUE) was grown anaerobically on fermentation broth under limited substrate concentrations in order to determine exponential growth period and to estimate optimal hydraulic retention time should be applied in continuous system. At first, the dependence of bacterial growth was investigated using different amount of formate in the reaction mixture. The results are shown in Fig. 2.

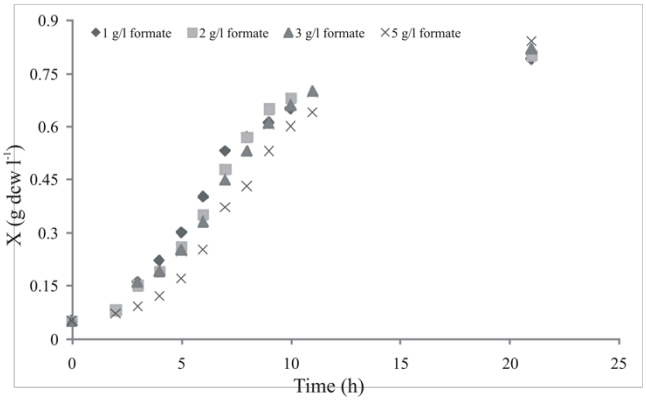


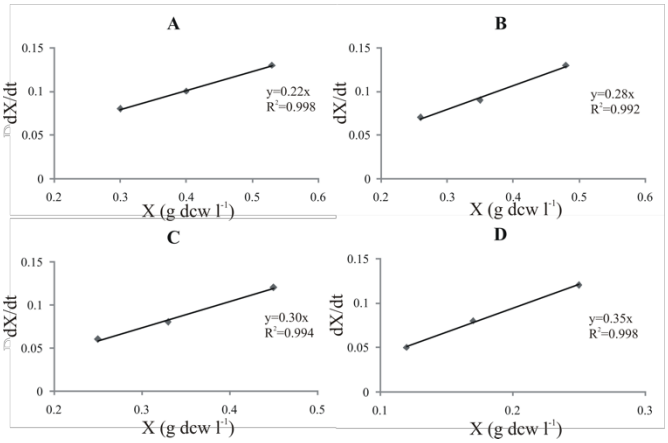
Fig. 2 – Time courses of bacterial growth using different formate concentrations

Theoretically, if the change of biomass concentration (dX/dt) is depicted as the function of biomass concentration (X) then linear correlation should be obtained in the exponential growth phase, and the specific growth rate (μ) could be estimated as the slope of the progress curve based on the following equation (Eq. 2):

$$dX/dt = \mu X$$

Biohydrogen production is basically associated with cell growth. Since the hydraulic retention time (HRT) and the biomass retention time (BRT) can not be set independently from each other in the CSTR configuration, it would appear useful to operate the bioreactor with a hydraulic retention time that ensures the highest microbial activity coupled with intense hydrogen production, which is expected to take place

till the end of the exponential phase of cell growth is reached. Moreover, the saturation constant (K_s) and maximum specific growth rate (μ_{max}) hold some physical significance. The kinetic constants can be used as design parameters for hydrogen producing bioprocesses with a variety of reactor configuration as well as in a CSTR. In all cases – except the experiment with 4 g/l formate which has been failed and therefore was not taken into account – linear relationship could be fitted to the experimental values obtained between 5-7 hours and subsequently it indicates that the exponential phase of bacterial growth takes place between the same time interval (Fig. 3).



A: 1 g/l formate; **B**: 2 g/l formate; **C**: 3 g/l formate; **D**: 5 g/l formate

Fig. 3 – Graphs on *dX/dt* data *vs. X* data

Specific growth rates were determined as the slope of the respective progress curves according to Eq. 2.

Determination of kinetic constants

As mentioned, it is important to estimate kinetic constants e.g. maximum growth rate (μ_{max}) and saturation constant (K_s). For instance, the μ_{max} value suggests that the continuous culture should not be carried out at dilution rates close to or above that, otherwise biomass wash-out could occur. In addition, the K_s represents the substrate concentration that is required to achieve 50% of the maximum growth rate and hence, it could be used as a guideline for adjusting the most efficient substrate concentration in the feed.

For these reasons, Lineweaver-Burk plots were used to obtain the kinetic parameters for cell growth with different concentrations of formate as the sole carbon source, where the reciprocal values of specific growth rates from Table 1 are plotted against the corresponding reciprocal values of substrate concentrations.

The double reciprocal equation can be described by the following equation (Eq. 3):

$$1/\mu = (K_s/\mu_{max})*(1/S) + 1/\mu_{max}$$

The graphical interpretation of Eq. 2 can be seen in Fig. 4 by using the experimental data from Fig. 3.

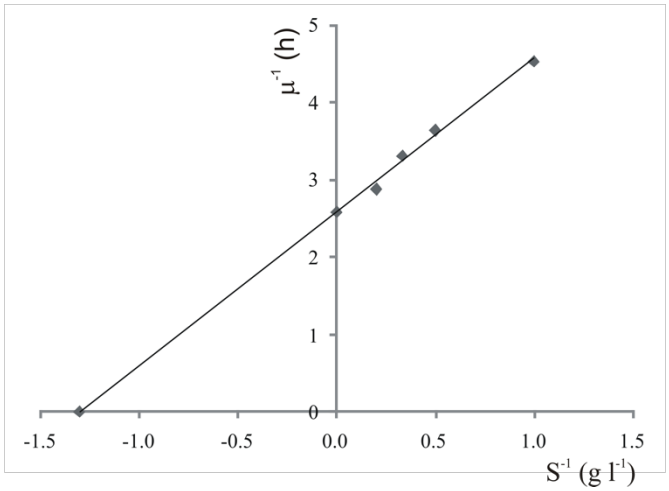


Fig. 4 – The double reciprocal plot for determining μ_{max} and K_s

In this research, the μ_{max} and K_s were found to be 0.39 h^{-1} and 0.77 g l^{-1}, respectively. This maximum specific growth rate is consistent with those reported in other study with other *E. coli* strains. Saturation of growth at relatively low carbon concentrations reflects the efficient substrate transport capability of *E. coli* (XL1-BLUE). The results imply that applying continuous cultures of *E. coli* (XL1-BLUE) for biohydrogen formation the hydraulic retention time in the bioreactor must be chosen above μ_{max}^{-1} (2.6 h) in order to prevent the wash-out of whole cell biocatalysts.

Investigation of the effect of HRT on continuous hydrogen production

The effect of HRT on hydrogen production was investigated using *E. coli* (XL1-BLUE) in a completely mixed tank reactor. As it was demonstrated in the previous section, the exponential phase of bacterial growth employing *E. coli* (XL1-BLUE) takes place between 5-7 h of the cultivation time. In addition, according to our preliminary work it was shown that bacterial growth and H_2 formation take place in paralell with each other. Recently, it has been reported that various hydrogenases in *E. coli* are induced at different times of strain cultivation. Moreover, formate-dependent hydrogen generation increases quickly from the beginning of the cell growth and reaches its maximum during the exponential growth phase. On the other hand, the activity of H_2-uptake enzymes rises rather slowly and reaches its highest value during the stacionary phase. Thus, it was supposed that operating the reactor with a HRT which does not allows that bacterial growth exceeds exponential phase could lead to enhanced biohydrogen formation. Therefore, experiments were performed by applying various hydraulic retention times related to the exponential (5 h, 7 h) and stacionary phase (9 h). The fermentation curves are presented in Fig. 5 - 7.

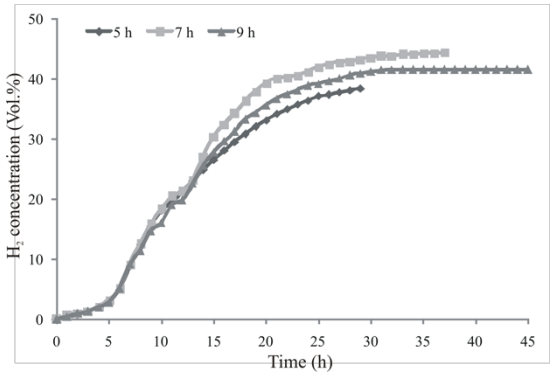


Fig. 5 – H_2 concentration curves for the different HRTs measured by BlueSens H_2 analyzer

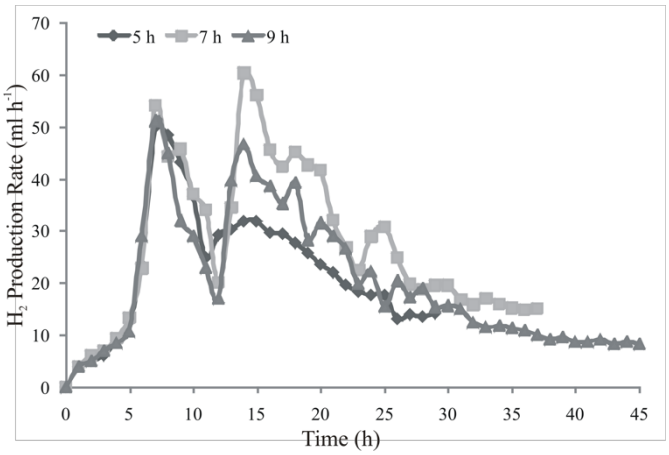


Fig. 6 – H_2 production rates for the various applied HRTs

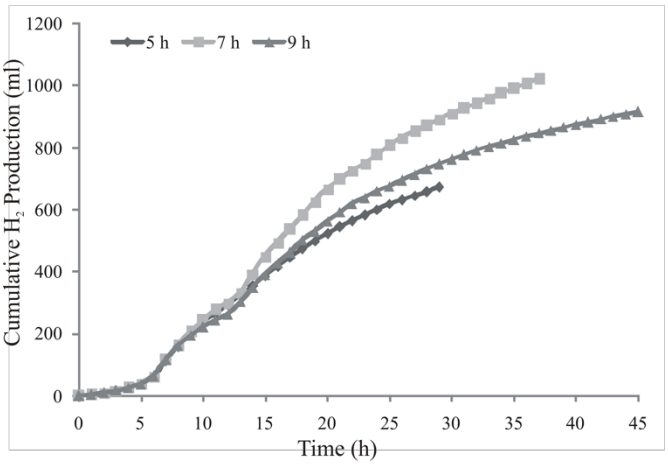


Fig. 7 – The time profiles of cumulative H_2 productions

As it can be seen in Fig. 6, the progress curves during the first 8 hours of batch operation are quite identical that represents the good reproducibility of the measurements. Fig. 6 also demonstrates when the reactor was switched to continuous mode the production rates were strongly decreased and then increased possibly since the cell growth was affected by batch-continuous transition that might also affect hydrogen production. After this transient state, the hydrogen production rates have more or less the same profiles in time, however the values are seemed to be influenced by the applied HRT. Fig. 6 clearly shows that the hydrogen generation was the most intense for HRT=7h followed by HRT=9 and HRT=5h. For the continuous biohydrogen process, the efficiency can be characterized by both hydrogen yield and productivity. Thus, a suitable dilution rate of a continuous hydrogen

producing bioreactor system should be determined by comprehensively taking both yield and productivity into consideration. Therefore, the results were compared based on the calculated hydrogen yield and productivity values which can be seen in Table 1.

Table 1 – The evaluation of the effect of HRT on biohydrogen production

		HRT (h)		
		5	7	9
Time of operation (h)	batch mode	8	8	8
	continuous mode	21	29	37
Number of cyles in continuous mode		4.2	4.15	4.1
Hydrogen produced (mmol)		27	40	36
Formate added (mmol)		156	155	153
Hydrogen Yield (mmol H_2 $mmol^{-1}$ Formate)		0.17	0.26	0.23
Hydrogen Productivity (mmol H_2 $l^{-1}d^{-1}$)		4.3	5.1	3.8

Dipl. Eng. Péter Bakonyi, University of Pannonia
Research Institute on Bioengineering, Membrane Technology and Energetics
Ph.D. Nándor Nemestóthy, University of Pannonia
Research Institute on Bioengineering, Membrane Technology and Energetics
Prof. Katalin Bélafi-Bakó, University of Pannonia
Research Institute on Bioengineering, Membrane Technology and Energetics

The main tasks of the Research Institute on Bioengineering, Membrane Technology and Energetics are:
- to teach and train students to get BSc in bioengineering
- to introduce them to a wide range of biochemical and biotechnological processes
- to introduce them to the world of membranes and their applications .

Application possibilities of various membrane processes, improvements of bioprocesses and manufacture of renewable, "green" energy sources are studied at the institute involving the PhD students of the Doctoral School of Chemical Engineering and Material Sciences (University of Pannonia).

According to Table 1, it can be concluded that hydrogen production – including both yield and productivity – increases with increasing the HRT, reaching a maximum value at HRT=7 h and then declines. Among the three studied hydraulic retention times, the highest hydrogen yield and productivity was 0.26 mmol H_2/mmol formate and 5.1 mmol H_2 $l^{-1}h^{-1}$, respectively at HRT=7 h. As a consequence, the results suggest that 7 h can be considered as the optimal value of hydraulic retention time for hydrogen production. This confirms the hypothesis which suggested that adjusting the HRT to the end of exponential phase could yield improved bioH_2 production.

Conclusion

The results indicated that *E. coli* (XL1-BLUE) is capable to grow and evolve biohydrogen in continuous cultures. The kinetic study of bacterial growth resulted in useful design parameters (μ_{max}, K_s) for the continuous hydrogen producing bioreactor. As shown here, the hydraulic retention time is an important factor that can influence the overall efficiency of continuous hydrogen fermentation. For these results, a real-time BlueSens hydrogen sensor was used, that has been appeared a very useful tool in the process optimization of fermentative hydrogen production.