Bioprocess Engineering 19 (1998) 205–211 © Springer-Verlag 1998

Experimental study of hydrogen kinetics from agroindustrial by-product: Optimal conditions for production and fuel cell feeding

P. Perego, B. Fabiano, G.P. Ponzano, E. Palazzi

Abstract One of the best and cleanest systems to produce electric energy is represented by fuel cells, whose natural fuel is hydrogen. In this paper, the production of hydrogen rich biogas is studied. This process contributes to create a system for biomass recovery, which eliminates organic pollutants and produces energy with high efficiency without atmospheric emissions. The study has been based on *Escherichia coli* and *Enterobacter aerogenes* strains. The research deals with batch reactors and verification of optimal conditions of hydrogen production. The realization of the optimal working conditions would conduce to the realization of a reactor suitable to feed a stack of the above mentioned fuel cells. In view of industrial applications, some different ways have been considered to greatly enhance the process performance, in terms of rate of hydrogen production, efficiency of hydrogen utilization and/or biosynthesis of valuable subproducts.

List of symbols

C	glucose concentration at the end of the fermentation (g l^{-1})
C_0	initial glucose concentration (g l^{-1})
n	hydrogen evolution (mmol)
n_{max}	maximum hydrogen evolutiion (mmol)
r_{max}	maximum rate of hydrogen evolution (mmol l^{-1} h^{-1})
$\hat{r}_{max}$	maximum specific rate of hydrogen evolution (mmol h^{-1} g^{-1}_{cells})
Y_{max}	maximum yield (mmol H_2 $mmol^{-1}_{glucose}$)

1 Introduction

Fuel cells can be considered to be one of the cleanest and most efficient systems to convert the chemical energy stored in several categories of substances, owing to their high conversion efficiency and environmental compatibility. Among these substances hydrogen plays a pre-eminent role, thanks to its good reactivity and high oxidation energy [1]. Moreover, hydrogen is an attractive energy source for replacing conventional fossil fuels, both from the economic and environmental standpoints [2]. In general, there are two ways to produce hydrogen with living organisms: one is hydrogen production by photosynthetic organisms and the other is fermentative hydrogen production. Several facultative and strictly anaerobic bacteria were used in the fermentation of different substrates [3–5]. The main advantages of the fermentative hydrogen production may be summed up as follows [6]:

- production for 24 hours a day without light;
- use of photosynthetic products as substrates;
- use of industrial and/or agricultural wastes as substrates;
- metabolites, except hydrogen, can also be used.

Moreover, as the rate of fermentative hydrogen production rate is usually higher than the photohydrogen evolution one, hydrogen bio-production via fermentation appears most likely the technique to be adopted in view of industrial application.

Fermentation of high BOD wastes or by-products seems to be a very promising way for producing hydrogen to be used in fuel cells, as it simultaneously allows a good recovery of energy and some valuable by-products, satisfying the need of environmental protection. To this purpose, research on economical feasibility of a continuous bioprocess suitable to feed phosphoric acid fuel cell (PAFC) has been undertaken, outlined as follows:

1) experimental study of the fermentation kinetics, carried out on laboratory scale, in order to find the optimal conditions for hydrogen production;
2) design, realization and operating of a packed bed continuous reactor (pilot plant scale) able to feed a 5 kW PAFC.

In the present paper we report the results of the laboratory scale tests and investigate the effect of operating conditions on hydrogen kinetics. As batch or fed-batch reactors generally allow the attainment of a higher final product concentration, we have chosen to study batch fermentation. As the interest of the present study was directed to industrial applications, the experimental phase of the work was planned as follows:

At first, the batch fermentation behaviour of synthetic and derived-waste substrates was investigated, in presence

Received: 7 October 1997

P. Perego, B. Fabiano, G.P. Ponzano, E. Palazzi
ISTIC – Institute of Chemical and Process Engineering "G.B. Bonino", University of Genoa, Via Opera Pia 15, I-16145 Genoa, Italy

Correspondence to: P. Perego

The authors wish to dedicate the present paper to Prof. Giuseppe Ferraiolo an unforgettable guide, master and scientist. He will always be remembered for his precious scientific skill, outstanding professional competence and, above all, for his incomparable human endowment.

of both *Escherichia coli* and *Enterobacter aerogenes* strains, without pH regulation of the culture broth.

Then, further experimental runs were performed in the neighbourhood of the optimal values of the process variables, as suggested from the previous experiments. The pH, in particular, was maintained at a constant level of 5.5.

2 Materials and methods

2.1 Microorganisms

Pure cultures of *E. coli* (NCIMB 11943) and *E. aerogenes* (NCIMB 10102), used for this study, were maintained on nutrient agar slants at 4 °C and subcultured monthly. The cells were then incubated aerobically respectively at 37 °C and 40 °C, in shake flasks on a rotary shaker at 150 rpm and harvested at the stationary phase.

The pre-culture medium consisted of 1.5% glucose, 0.2% yeast extract, 0.5% peptone, 0.5 NaCl, 0.1% beef extract, all in weight percent; the cells were aseptically inoculated into the fermentor 12 h after they had been harvested.

2.2 Media

Some preliminary tests were carried out with a synthetic medium, *S*, consisting of 1.5% glucose, 0.5% yeast extract, 1.4% KH_2PO_4, 0.2% $(NH_4)_2SO_4$, 0.1% sodium citrate dihydrate and 0.02% $MgSO_4 \cdot 7H_2O$, all in weight percent. Further tests were performed utilizing, as a waste-derived medium, a corn starch hydrolisate, *H*, rich in glucose and natural compounds important for microbial growth, kindly supplied by Roquette Italia SpA of Cassano Spinola (Italy). This was prepared by preliminary treating the corn starch with a heat-resisting α-amylase, until a concentration of 15–20% ED (equivalent dextrose) was obtained; a subsequent saccharification at 60 °C with amyloglucosidase, lead to an almost complete hydrolysis of the starch, as shown in Table 1.

The medium used for the batch runs was obtained by diluting corn starch hydrolysate with tap water, until the desired sugar concentration was achieved, and by adding the following salts: 6.0 g/l KH_2PO_4, 14 g/l K_2HPO_4, 2.0 g/l $(NH_4)_2SO_4$ and 0.4 g/l $MgSO_4 \cdot 7H_2O$; sodium citrate dihydrate and NaOH were used to buffer the medium at pH = 5.5.

2.3 Operative conditions

A 2.0 l Gallenkamp FBL-195 bioreactor with a working volume of 1.5 l, stirred at 150 rpm, was employed.

The pH of the fermentation broth was automatically controlled, with an accuracy of 0.1 pH unit, by a pH control module FBL-725 provided with a peristaltic pump which injected a fine stream of 30% NaOH solution. The internal atmosphere was saturated with nitrogen before starting each experimental run. The nitrogen flow rate was set at 1 l/min and controlled by a Bronkhorst Hi-Tec flowmeter.

The temperature was kept at a constant value of 37 ± 0.5 °C for *E. coli* and of 40 ± 0.5 °C for *E. aerogenes* by a temperature control module FBL-360.

The fermentor and the medium were sterilized by autoclaving at 120 °C for 20 min; there was no evidence of contamination in any of the fermentations carried out during this study.

A schematic diagram of the experimental set-up is shown in Fig. 1.

Table 1. Composition of the strach hydrolisate

Component	Amount
Dry extract	69.5%
Glucose	85.0% of dry extract
Maltose	2.6% of dry extract
Trisaccharides	0.7% of dry extract
Oligosaccharides	6.85% of dry extract
Ashes	3.8% of dry extract
Protein (N · 6.25)	1.05% of dry extract
Ca^{2+}	0.305 mg g^{-1}
Mg^{2+}	0.129 mg g^{-1}
Na^{+}	71.50 mg g^{-1}
K^{+}	1.09 mg g^{-1}

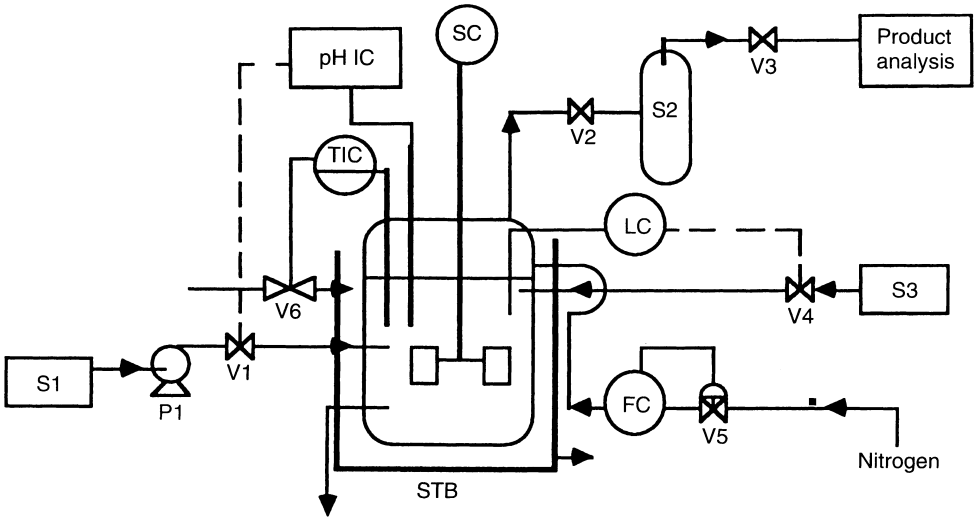

Fig. 1. Experimental set-up: STB stirred tank bio-reactor, S_1 NaOH storing vessel, S_2 effluent gas sampling system, S_3 water storing vessel, SC stirring controller, LC level controller, FC flowmeter, TIC temperature indicator and controller

2.4 Product analyses

During the batch runs, the hydrogen evolution rate was measured by means of a gas chromatograph Perkin-Elmer Sigma 3, (Milano, Italy), equipped with a thermal conductivity detector. Nitrogen at 3.5 bar was used as carrier gas. The column employed was a 2 m long tube of 1/8″ diameter, packed with 80–100 mesh Chromosorb 102 support.

Acetic acid, propionic acid, lactic acid and ethanol were measured by a gas chromatograph Shimadzu 14A equipped with a flame ionization detector and a 2 m × 2 mm ID glass column packed with 80/120 Carbopack B-DA/4% Carbowax 20 M (Supelco, Bellefonte, PA). The column temperature was set at 175 °C and the injector and detector temperatures were both set at 200 °C, the carrier gas was helium.

Glycerol was measured by a Knauer HPLC Carlo Erba CPS4 (Carlo Erba, Milano, Italy), equipped with a 30 cm × 7.8 mm ID Supelchem column packed with Supelcogel Ag (Supelco, Bellefonte, PA), employing H_2O as mobile phase.

2.5 Biomass analysis

The cell concentration was determined by filtering a known volume of culture broth with 0.2 μm autoclavable filters. The filters were dried at 105 °C, until no weight change between consecutive measurements was observed.

All the fermentations were carried out with an inoculum of 1.34 g dry weight (dw) l^{-1}. A few milliliters of a thick suspension of the microorganism, obtained by aseptic centrifugation, whose biomass concentration was previously determined as reported above, was added to the medium until the desired initial biomass concentration was attained.

2.6 Substrate analysis

The glucose concentration as well as the content of the starch hydrolysate during the batch runs, were determined by means of the Boehringer enzymatic method Cat. N° 716251 [7].

The maltose content was calculated by the same method, after hydrolysis of glucose by using the enzyme α-glucosidase, in compliance with the Boehringer method.

Raw proteins were determined by the Kjeldahl method [8], and metallic ion concentrations by atomic absorption spectrophotometry [9], utilizing a Perkin Elmer 5000 Atomic Absorption Spectrophotometer (Monza, Italy).

3 Experimental results

3.1 Fermentation behaviour without pH control

We have performed 11 batch fermentation tests without pH control, according to the methods previously indicated.

In Table 2 are reported the utilized microorganisms, substrates types and concentrations, together with the most significant results obtained from the experimental data. Each experimental result represents the average of three replicates, with differences not exceeding 4%. During fermentation runs of the synthetic medium *S*, four different initial glucose concentration S_0 were tested for *E. coli* strain, namely 2, 5, 10 and 18 g l^{-1}.

Hydrogen evolution (Fig. 2), cell growth (Fig. 3) and glucose consumption (Fig. 4) clearly show that the time required for complete batch runs gradually increases with

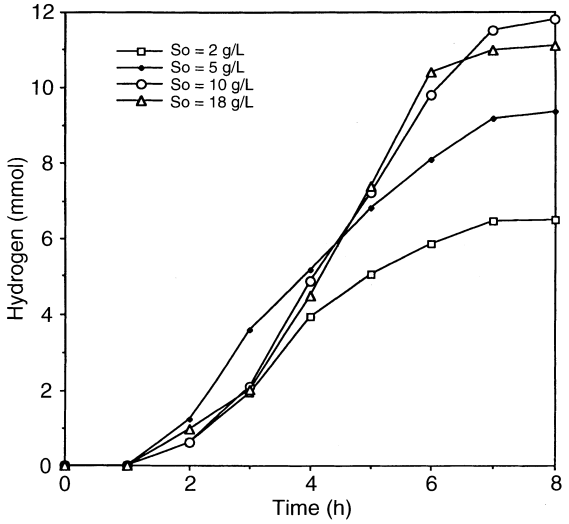

Fig. 2. *E. coli* total hydrogen evolution on glucose synthetic substrate *S*

Table 2. Hydrogen evolution without pH control

Run	Microorganism	Substrate		*n* [mmol]	r_{max} [mmol l^{-1} h^{-1}]	*Y* [mmol H_2 $mmol^{-1}_{glucose}$]	$\hat{r}_{max}$ [mmol h^{-1} g^{-1}_{cells}]
		Type	C_0 [g l^{-1}]				
1		*S*	2	6.4	1.3	0.43	0.59
2		*S*	5	9.2	1.5	0.40	1.25
3	*E. coli*	*S*	10	11.6	1.8	0.25	0.80
4		*S*	18	10.5	2.4	0.16	0.81
5		*H*	10	14.4	2.1	0.36	1.75
6		*H*	18	12.6	1.9	0.26	1.52
7		*S*	10	12.2	2.0	0.30	1.96
8		*S*	18	7.2	2.0	0.19	2.38
9	*E. aerogenes*	*H*	5	45	5.2	1.8	4.73
10		*H*	10	42	5.3	1.1	4.14
11		*H*	18	58	5.3	0.63	3.31

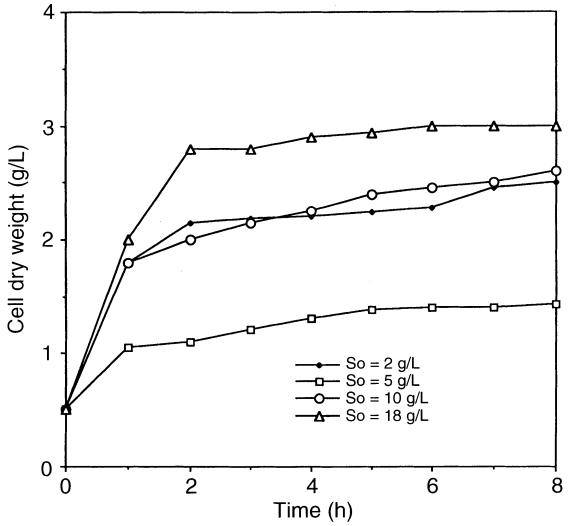

Fig. 3. *E. coli* cell growth on glucose synthetic substrate *S*

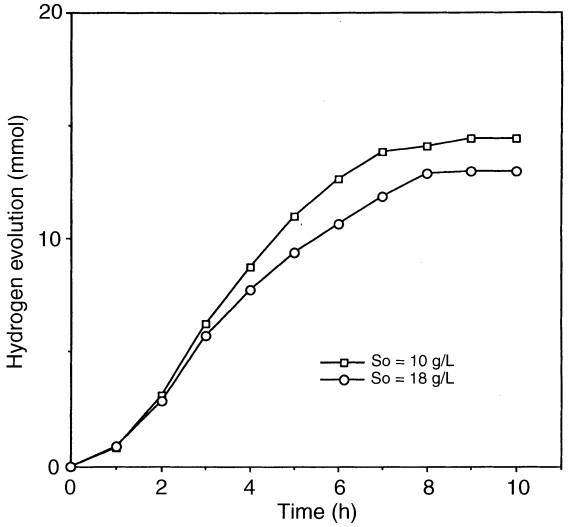

Fig. 5. *E. coli* total hydrogen evolution on starch hydrolysate *H*

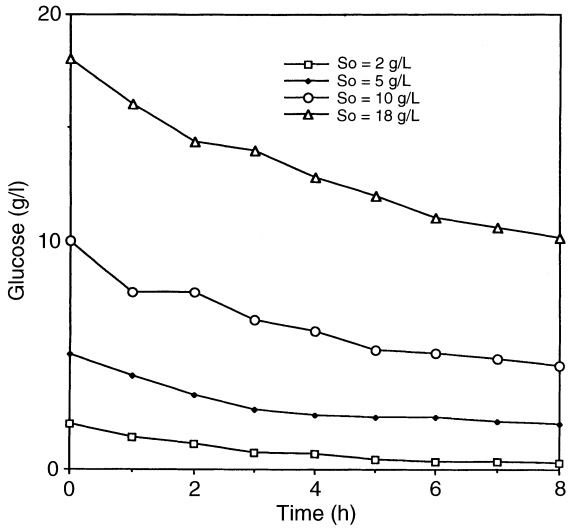

Fig. 4. *E. coli* glucose consumption on glucose synthetic substrate S

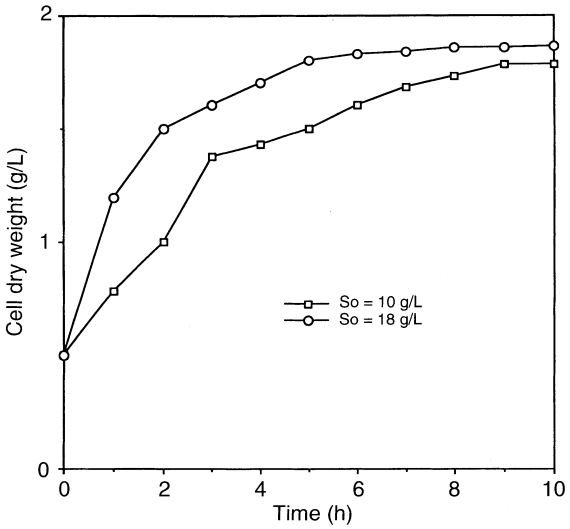

Fig. 6. *E. coli* cell growth on starch hydrolysate *H*

increasing initial substrate concentration. At substrate concentration higher than 18 g l^{-1}, we observed an inhibition phenomenon and subsequent incomplete glucose degradation.

Similar effects have been observed in the behaviour of hydrogen evolution, cell growth and glucose consumption during fermentation runs performed by *E. coli* strain on starch hydrolisate, respectively reported in Figs. 5, 6 and 7.

To draw conclusions, all experimental results are listed in Table 2, referring to batch fermentations of the two different substrates *S* and *H*, employing *E. coli* and *E. aerogenes*. The results show a typical behaviour of cell growth, hydrogen production and glucose consumption, namely:

- in the first two hours a very high glucose consumption and cell growth take place, without hydrogen production;
- in the next 2–4 hours, glucose consumption and cell growth slow down, while hydrogen generation vigorously increases;

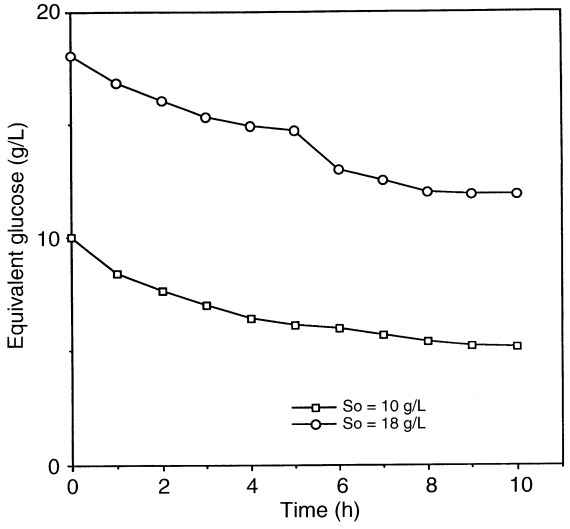

Fig. 7. *E. coli* glucose consumption on starch hydrolysate *H*

– finally, in the period of a few hours, cell growth and glucose consumption become meaningless and hydrogen production rate slowly decreases until zero.

The pH decreases rapidly, with bacteria growing, then slowlier, up to reach a value of 5.3–5.4 and 4.5–4.7, respectively in fermentations carried out by means of *E. coli* and *E. aerogenes*. The incomplete substrates utilization can be attributed to some acid by-products, which inhibit the microbial metabolism [10].

3.2
Hydrogen production without pH control

With reference to Table 2, a comparison on the efficiency of the hydrogen production by the utilized microorganisms and substrates can be made.

Independent of the glucose concentration, the best results have been obtained with fermentation of starch hydrolysate, *H*, by means of the *E. aerogenes* strain:

– the maximum hydrogen evolution, $n_{max} = 58$ mmol, and the maximum rate of the hydrogen evolution, $r_{max} = 5.3$ mmol l^{-1} h^{-1}, have been observed in the experimental run 11, performed with the maximum initial concentration of the substrate, $C_0 = 18$ g l^{-1};
– the maximum yield, $Y_{max} = 1.8$ mmol H_2 $mmol^{-1}_{glucose}$, and the maximum specific rate of hydrogen evolution, $\hat{r}_{max} = 4{,}73$ mmol H_2 h^{-1} g^{-1}_{cells}, took place in the experimental run 9, where the most diluted substrate was utilized, $C_0 = 5$ g l^{-1}.

In general the values obtained for *E. aerogenes* are higher than the ones obtained for *E. coli*, showing a better performance of the former strain.

Furthermore, the employment of the synthetic substrate S shows lower performance in terms of the maximum rate of hydrogen evolution and of the maximum specific rate of hydrogen evolution, probably owing to the presence of important growth factors in the starch hydrolysate.

The maximum rate of the hydrogen evolution was observed about in the fourth hour.

The aforesaid results agree with those obtained by Tanisho et al. [6], which however operated only on 30 ml cultivations, using a synthetic medium.

3.3
Fermentation behaviour with pH control

Following the previous experimental runs, we have performed another six batch fermentations, with *E. aerogenes* and relatively high starch hydrolisate concentrations, trying to optimize both the hydrogen production and its rate.

In each test the pH, after a quick decrease from about 6.2 to 5.5, was maintained at this value up to the end of the process, by injecting into the fermenter about 4 cm^3 h^{-1} of the aforesaid 30% NaOH solution. To obtain some indications on the prevailing fermentation pathways, also the concentrations of the most significant organic end-products have been determined. The main features of these experiments are reported in Tables 3 and 4.

Each experimental result represents the average of three replicates, with differences not exceeding 4%. In comparison with the experiments at uncontrolled pH, the behaviour of the cell growth, hydrogen production and glucose consumption is more complicated, as shown in Figs. 8, 9 and 10.

Since the pH control avoids microorganism inhibition, the glucose consumption is nearly complete in each fermentations. However, particularly at higher substrate concentrations, a kind of different metabolic pathway seems to become active after the first 6–7 hours, which drastically reduces the rates of the glucose consumption and the hydrogen production, up to a mean value of about 2 g l^{-1} h^{-1} and 2.5 mmol l^{-1} h^{-1}, from now to the end of fermentation.

Moreover, as reported in Table 4, the high substrate concentrations seem to promote the formation of the most reduced metabolite end products, as glycerol, ethanol and 2-3 butanediol.

Finally, the oscillating behaviour of the fermentation rate leads to a conclusion that no substrate limitation af-

Table 3. Hydrogen production under controlled pH

Run	Microorganism	Substrate		n [mmol]	r_{max} [mmol l^{-1} h^{-1}]	Y [mmol H_2 $mmol^{-1}_{glucose}$]	$\hat{r}_{max}$ [mmol h^{-1} g^{-1}_{cells}]
		Type	C_0 [g l^{-1}]				
12		*H*	18	150	10.7	1.1	6.3
13		*H*	18	141	14.6	0.54	5.3
14	*E. aerogenes*	*H*	18	148	14.9	0.60	8.0
15		*H*	36	133	10.3	0.45	3.0
16		*H*	36	127	11.3	0.34	3.6
17		*H*	54	125	8.2	0.28	2.9

Table 4. Concentrations of some significant metabolites end-products at the end of fermentation (g l^{-1})

Run	Substrate		Glycerol [g l^{-1}]	Acetic acid [g l^{-1}]	Lactic acid [g l^{-1}]	Propionic acid [g l^{-1}]	Ethanol [g l^{-1}]	2-3 Butanediol [g l^{-1}]
	C_0	C [g l^{-1}]						
14	18	0.05	0.45	1.90	1.40	0.61	1.84	2.54
15	36	7.25	0.47	1.10	2.96	1.07	1.12	7.74
17	54	10.9	0.81	1.14	2.44	1.23	2.89	7.84

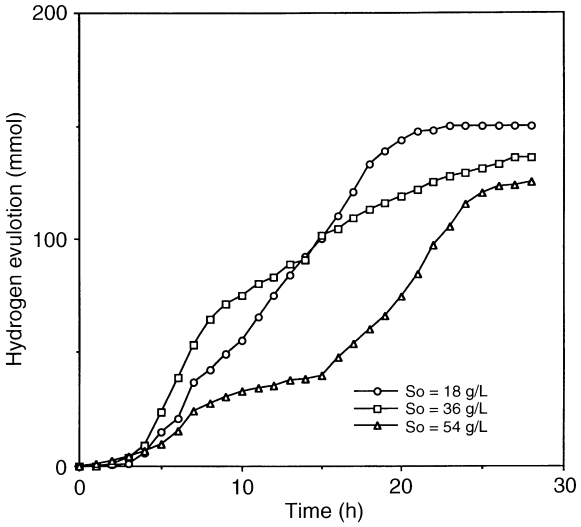

Fig. 8. *E. aerogenes* total hydrogen evolution under controlled pH on starch hydrolysate *H*

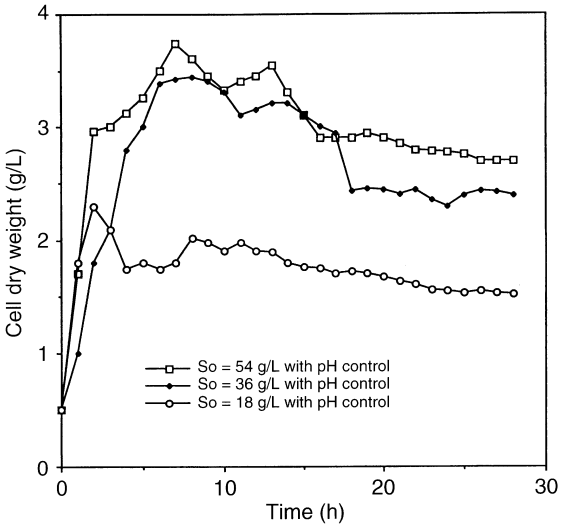

Fig. 9. *E. aerogenes* cell growth under controlled pH on starch hydrolysate *H*

fects the culture broth, until the glucose concentration remains above 1–2 gl^{-1}.

3.4
Hydrogen production with pH control

The experimental values of the most interesting parameters related to the hydrogen production are reported in Table 3. The best results have been obtained with an initial concentration of the substrate, $C_0 = 18$ g l^{-1}. In particular:

- the maximum hydrogen evolution, $n_{max} = 150$ mmol, and the maximum yield, $Y_{max} = 1.1$ mmol H_2 $mmol^{-1}_{glucose,}$ have been observed in the experimental run 12;
- the maximum rate of the hydrogen evolution, $r_{max} = 14.9$ mmol l^{-1} h^{-1} and the maximum specific rate of the hydrogen evolution, $\hat{r}_{max} = 8.0$ mmol H_2 g^{-1}_{cells} h^{-1} have been found in run 14.

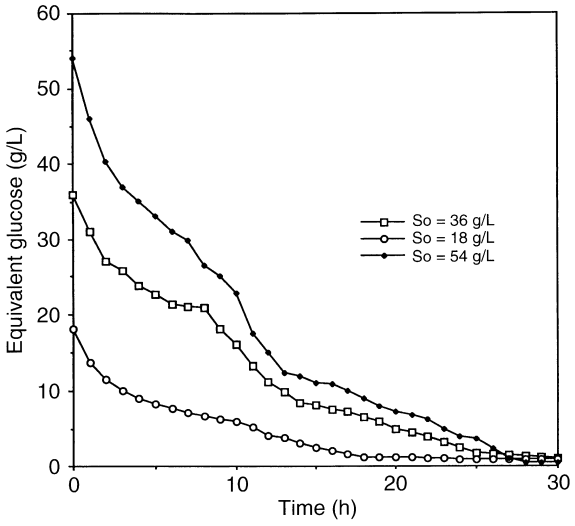

Fig. 10. *E. aerogenes* glucose consumption under controlled pH on starch hydrolysate *H*

- the maximum rate of the hydrogen evolution was observed within the fifth and the seventh hour.

The comparison of hydrogen evolution by *E. aerogenes,* with and without pH control, is reported in Fig. 11. The initial glucose concentrations have been 18, 36 and 54 g l^{-1}.

4
Discussion

4.1
Optimal operating conditions for a continuous fermentation process

From the experimental results described in the latter section, some important conclusions can be drawn, in order to foresee the optimal operating conditions for a contin-

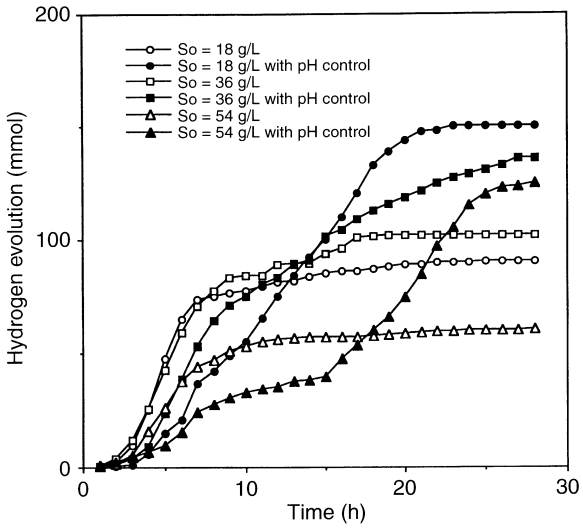

Fig. 11. Comparison of *E. aerogenes* total hydrogen evolution under controlled and uncontrolled pH on starch hydrolysate *H*, with initial substrate concentration corresponding to 18 g l^{-1}; 36 g l^{-1} and 54 g l^{-1}

uous fermentation process. As pointed out by Pauss et al. [11], the dissolved hydrogen concentration could be higher than that in equilibrium with the hydrogen partial pressure in the gaseous phase, owing to the relatively low rate of hydrogen transfer from the liquid to the gas phase, in comparison with the biochemical process of the hydrogen production and reutilization.

To approximately quantify the extent of the hydrogen-consuming reactions, the data reported in Table 4 and the ones on pH control can be considered. It results that the evolved hydrogen is about only one third of that produced in the earlier fermentation steps. From this point of view, the initial glucose concentration should have an essential influence on the yield and production rate of the hydrogen evolved from the liquid broth. With relatively low initial glucose concentrations, the rate of the earlier fermentation steps was also low, according to the mass action law. Nevertheless, an even more slow secondary metabolites production and hydrogen consuming reactions take place, so a large part of the dissolved hydrogen is able to pass into the gaseous-phase. Thus, a relatively high hydrogen yield can be obtained, notwithstanding the low production rates.

Increasing the initial glucose concentration, the fermentative process becomes in general faster, so the dissolved hydrogen concentration also increases, and, with it, the rate of the hydrogen evolution. Yet, the yield rapidly decreases, since the hydrogen biochemical reutilization grows even more. At very high initial substrate concentrations, the relative importance of the various fermentation pathways substantially modifies, so that both the hydrogen evolution rate and yield decrease. Coming to quantitative considerations, the optimal substrate concentration for a continuous process of this kind seems to range between 15–20 g l^{-1}.

By means of a most refined optimization procedure for a continuous process a specific production rate of the order of 10 mmol H_2 g_{cells}^{-1} h^{-1} should be obtainable.

4.2
Economical feasibility of the process

Before designing a continuous laboratory-scale fermentation reactor, some considerations on the economical feasibility of the whole process on industrial scale must be done.

Under the assumption of fuel cell conversion efficiency corresponding to 80% and operating in the range of the previously determined optimal conditions, a CSTR having a working volume of 5 m^3, with a concentration of 2 g_{cells} l^{-1}, will be needed to feed a 5 kW PACF, which is the target of this study.

Such a kind of process does not seem to be very attractive, neither with regards the energy recovery, nor from the point of view of the waste treatment. However, there are many favourable aspects that encourage the continuation of our research. Firstly, the enzymes concentration and then the rate of the hydrogen production could be increased of at least an order of magnitude, by adopting a suitable immobilization technique. Secondly, the possibility of merging electrodes of fuel cell into culture broth will be tested, aiming to greatly enhance both the yield and the utilization rate of the hydrogen, by subtracting it to the biochemical conversion steps [12]. Finally, some experiments will be carried out, to verify the opportunity to direct the continuous process, so as to promote the formation of some valuable fermentation end products, e.g. 2-3 butanediol, together with the hydrogen production [13–15].

5
Conclusions

In the fermentation of high BOD wastes or by-products, for hydrogen production, *E. aerogenes* proves more suitable than *E. coli*.

For a CSTR fermentor operating as follows: $T = 40$ °C, pH = 5.5, glucose concentration $C = 15$–20 g l^{-1}, a maximum hydrogen specific evolution rate of about 10 mmol h^{-1} g_{cells}^{-1} could be obtained.

In view of industrial applications, some different ways have been individualized to greatly enhance the process performance, in terms of rate of hydrogen production, efficiency of hydrogen utilization and/or biosynthesis of valuable subproducts. Our experimental research will pursue these lines.

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