Chapter 4
Effect of Temperature on Fermentative H_2 Production by HPB

In this chapter the analysis of the temperature effect on bioH_2 production is taken into account. Temperature is a very important factor, because it can affect the activity of HPB by influencing the activity of some essential enzymes such as hydrogenase for fermentative H_2 production [1], but at the same time it determines strongly the overall energy expense, hence the net energy balance. The first section is dedicated to the realization of a test at ambient temperature, which is exposed to natural night and day temperature variations, whereas the second section describes a series of tests at fixed temperatures (ranging from 16 to 50 °C). Each experimental test was conduced in a bench stirred-tank reactor.

4.1 Temperature: A Key Factor in Anaerobic Digestion

Among the operational conditions at which to run a bioreactor, the temperature, together with the pH, represents the most important parameter. The intensity of microbial activity on which the production of H_2 occurs depends on temperature. There are three possible ranges of temperature at which the process can be carried out: psychrophilic (15–25 °C), mesophilic (35–40 °C) and thermophilic (50–55 °C). For biogas production, only the mesophilic and thermophilic ranges are used in operational anaerobic digestion (AD) systems [2]. Generally speaking, this means that digesters run at around 35 and 55 °C respectively, though in absolute terms the optimal temperature may depend on the exact type of biowaste material undergoing treatment. Any AD process for biogas production requires a relatively constant temperature to progress at its greatest efficiency [2]. However, nowadays there is no industrial AD plant experience for H_2 production and therefore it is interesting to know the range of H_2 production according to temperature. Acidogenic bacteria seem to have a greater capacity to adapt to environmental variations such as temperature. In any case, the temperature controlling the nature of the microbial consortium is recognized by several authors: at 35 °C *Clostridium* are the dominant species, whereas at 55 °C lactic acid bacteria are more abundant [3]. For this reason, we conduced first of all a test at ambient temperature exposed to night and day

B. Ruggeri et al., *$BioH_2$ & $BioCH_4$ Through Anaerobic Digestion*,
Green Energy and Technology, DOI 10.1007/978-1-4471-6431-9_4

variation under natural decrease of pH in order to determine the flexibility of the microorganisms involved, and, subsequently, a series of tests at controlled temperature and pH in order to evaluate the hydrogen yield according to the working temperature. Very few studies have been conducted on the acetogenesis process at low temperatures. Lee and Chung [4] reported a study on H_2 production at 30 °C showing that there is microbiology activity at low temperature, even if the production rate at 30–34 °C (359 mmol H_2 L^{-1} h^{-1}) is almost 50 times greater than that at 15–18 °C. Mu et al. [5] conducted tests from 33 to 41 °C, finding that glucose degradation efficiency, H_2 yield and growth rate increased with increasing temperature from 33 to 39 °C, then decreased as the temperature was further increased to 41 °C. The distribution of aqueous products was also greatly influenced by temperature variation. In contrast, Akutzu et al. [6] found that hydrogen was successfully produced under thermophilic conditions and unstable H_2 production was observed in mesophilic conditions. The experimental results of Zhang and Shen [7] show that the hydrogen-producing ability of anaerobic bacteria was strongly affected by either culture temperature or iron concentration. The increase in the culture temperature facilitated hydrogen production when it was in the range of 25–40 °C and high sucrose conversion efficiencies (ca. 98 %) were consistently obtained; when the temperature rose towards 45 °C, however, hydrogen production was strongly inhibited. On the basis of these contrasting results and in order to better understand the influence of temperature, we conducted batch tests in a laboratory-scale reactor using anaerobic wastewater sewage sludge as inoculum, treated with HCl as reported in Chap. 2, in a glucose-rich medium with a concentration of 58 ± 2 g/L, according to the kinetics study described in Chap. 3.

4.2 Material and Test Procedure

The following section describes the method used for carrying out the tests. The tests were conducted in the same reactor, with the same medium and initial glucose concentration, monitoring the same parameters. The differences between tests concerning the bioreactor operation were:

- a single test was conducted without temperature and pH control, i.e. at ambient temperature and with the pH adjusted after natural decrease, in order to prove the dynamics evaluated in Chap. 3;
- a series of tests was then conducted under temperature and pH control.

4.2.1 Apparatus and Operative Conditions

The sludge was collected from an anaerobic wastewater treatment plant and pretreated as reported in the previous Sect. 2.3.1. The treated sludge was inoculated in

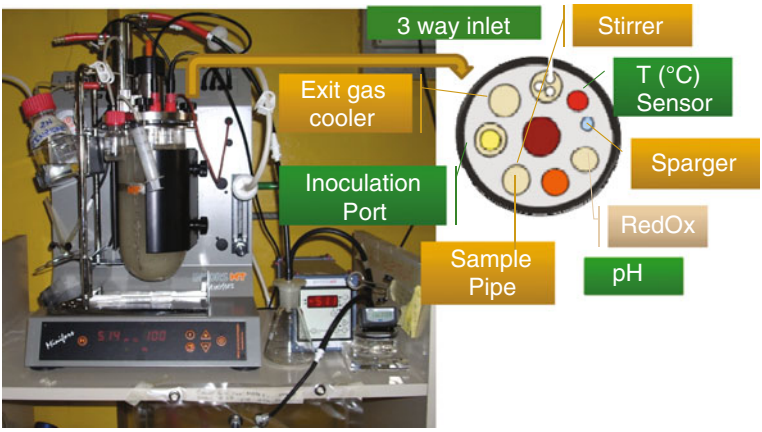

Fig. 4.1 Schematic view of bioreactor with various components used in the tests

a ratio of 10 % v/v in a stirred-batch reactor STR (Minifors HT, Switzerland), shown in Fig. 4.1, with 2 L as the working volume, running under anaerobic conditions obtained by flushing with nitrogen gas at the beginning of the fermentation, and by mixing the broth at 100 rpm. The reactor is equipped with a mixer, pH and temperature control systems, in order to easily check and change the internal conditions during hydrogen production. The above parameters were monitored continuously, in addition to the redox potential. The initial glucose concentration was 58 ± 2 g/L and the medium had the macro- and micro-nutrient composition described in Methodology in Chap. 2.

4.2.2 Test with Bioreactor

4.2.2.1 Test at Natural Ambient Temperature

The initial pH of the media was set at 7.5 and the initial C/N ratio was about 30. The experiment was carried out at ambient temperatures, which ranged from 23 ± 0.1 to 28 ± 0.1 °C. At the beginning of the test the fermentation was conducted without any pH regulation. After about 60 h, when the biogas evolution shut down (Fig. 4.2), the pH was adjusted with 2N NaOH at different times during the fermentation to verify if biogas production restarted. To be precise, pH was adjusted at pH 6.3 and pH 5.75 at times t = 67 h and 143 h, respectively, while at time t = 183 h it was set at pH 5.

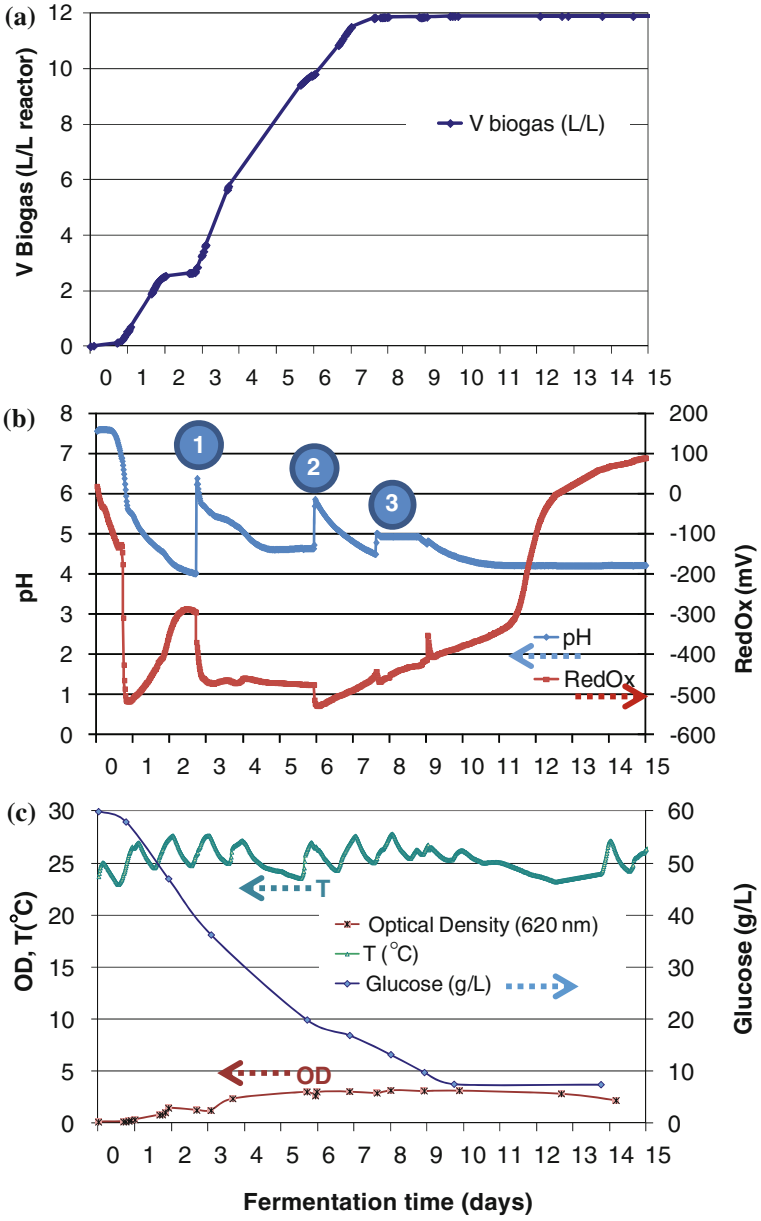

Fig. 4.2 **a** Cumulative biogas (H_2 and CO_2) production, **b** pH and redox, **c** optical density, temperature and glucose concentration

4.2.2.2 Test at Controlled Temperature

Batch tests for hydrogen production were conducted at different temperatures: 16, 20, 35, 40 and 50 °C (±0.1 °C) with the same glucose concentration as the test reported in Sect. 4.2.2. For each test, the initial pH of the medium was set at 7.2 ± 0.1. The first hours of fermentation were conducted without any pH regulation; after a natural decrease to 5.2, it was maintained at this value by 2N NaOH dosage by peristaltic pump and pH meter sensor probe. Tests were stopped after the biogas production shut down.

4.2.3 Monitored Parameters

Temperature, pH and redox potential were monitored online every minute through a data acquisition system (Iris NT Software; Infors AG, Switzerland). The gas produced during the fermentation was constantly measured with a volumetric gas counter (Milligascounter, Ritter) and then was collected in Tedlar bags (SKC, 231-05 series) and analyzed with a gas chromatograph (Varian, CP 4900). The liquid samples were taken out of the reactor daily at different times during the fermentation for the determination of glucose content, biomass concentration by optical density (OD) and volatile fatty acid (VFA) concentration using a gas chromatographic (GC) method. The GC was equipped with columns (polyethylene glycol ester, 0.5 m × 0.53 mm as precolumn and Alltech Superax-FABP, 25 m × 0.53 mm, Banded FSOT), the working temperature was 200 °C at detector, H_2 gas was used as carrier; the sample was filtered (0.45 μm) under vacuum, acidified (1 N HCl) and diluted with distilled water before injection.

4.3 Results of the Test at Ambient Temperature

4.3.1 Dynamics of Parameters Monitored

As a result of acetogenic fermentation, 1 L of glucose medium produced approximately 12 L of gas containing, on average, a hydrogen concentration ranging from 29 to 45 % (v/v) and carbon dioxide from 55 to 71 % (v/v). These values of H_2 content in the biogas under ambient temperature conditions were lower than those obtained in mesophilic conditions. The three graphs (a, b, c) of Fig. 4.2 provide an overview of the parameters monitored during the fermentation time. The first graph (4.2a) shows the amount of biogas (H_2 and CO_2) developed after 18–20 h (lag phase), the time during which the microorganisms reorganize their molecular constituents to adapt to new environmental conditions, as stated and commented in Chap. 3.

At first glance, from Fig. 4.2b we observe a strict relation between biogas evolution, pH and redox. During the lag phase (without pH control), between $t = 0$ and $t \sim 20$ h, pH slightly decreases from 7.5 to 6, while redox remains at very slightly reductive values; just before the beginning of the exponential phase, redox sharply decreases, reaching a minimum value of −518 mV, and subsequently, when the exponential phase of biogas production begins, pH falls and redox gradually starts to rise towards oxidative values. When the pH reaches 4.3, biogas production stops. This observation has been noted in other tests described in Chaps. 2 and 3, and also reported in several studies by other authors [8].

Bearing in mind that biogas production stops at pH 4.3, the pH was adjusted from 4.3 to 6.3 (point 1 in Fig. 4.2b), and then the exponential phase restarted and at the same time redox fell dramatically towards sharply reductive values. The same behaviour was also observed for points 2 and 3. At point 2, the pH was adjusted to 5.75; instead, at point 3, pH was set at 5.2, but only a small quantity of produced gas was detected. During biogas development, the redox moves gradually towards non-reducing environment conditions to oxidize the substrate and consequently also unfavorable conditions for producing H_2. Chen et al. [8] also found that at pH 4.5 the biogas production shut down in batch tests at 37 °C with HPB obtained by heat pretreatment, but an elevation of pH from 4.5 to 6.5 restored hydrogen production from the culture, suggesting that pH control at an appropriate level can improve the efficiency of hydrogen production, as stated in Chap. 2 from a biological point of view.

Glucose utilization reached a percentage of 77 % w/w, while the maximum rate of glucose consumption was 0.39 g glucose L^{-1} h^{-1}, detected between 18–46 and 74–137 h, during the exponential phases. The rate of glucose consumption decreased at the same time at which the biogas production rate also dropped, although the pH was set at 5.2 (point 3). Probably microorganisms stressed from pH variations were no longer able to adapt to the new conditions, especially since the pH was adjusted after about 3 h when the bacteria were in unfavorable conditions, i.e. pH < 4.5. This test confirms that the optimal pH for anaerobic H_2 production reported in the literature is essentially within the range 5.2–6.5 [8–10]. This suggests that ensuring a more than satisfactory control loop for pH control in a scaled-up system of biohydrogen production is of utmost importance. HPB demonstrate a great ability to adapt to environmental variations, such as temperature which fluctuates in the range 23–27 °C according to the uncontrolled ambient conditions test (Fig. 4.2c); in addition the pH variation in the range 4.5–6 seems not to suppress HPB activity, even though HPB remaining at low pH values for about 2 h have shown difficulties in reactivating the H_2-producing pathways. Figure 4.2c shows that there was a cell growth of saturation type. The biomass increased in the first 2 days since the fermentation began, thereafter it remained stable and decreased steadily only during the stationary H_2 production phase, suggesting that the hydrogen production was coupled with the stationary phase of the growth curve of the bacterial population. This consideration is in accordance with the study of Chen et al. [8], where H_2 evolution appeared to start after the middle stage of the exponential phase, with maximal H_2 production when cell growth had entered an

early stationary phase. This seems to imply that H_2 generation was not a preferable event during assimilation of the carbon substrate for gain in biomass; in fact the predominance of metabolic electron flow toward biosynthesis decreases the availability of electrons for hydrogenase to produce H_2, as seen in the previous Chapters. This aspect suggests that it is necessary to increase the concentration of microorganisms per unit volume in order to increase the volumetric production of hydrogen, for example by using a fixed-bed reactor with immobilized and/or entrapped HPB. Unfortunately this type of bioreactor is only suitable for liquids having very low substrate concentration (total solids less than 2–3 %).

From the above comments, it is possible to conclude that gas production strongly depends on pH and on high reductive redox level. Moreover, an interesting relationship between pH and redox was observed (Fig. 4.2b) throughout the performed tests. This confirms the hypothesis of the two species of H^+ ions present in the medium: one involved in the biological pathway linked to Fd and hydrogenase pool of enzymes, and the other one like protons, acting in the water domain to assure the electrical neutrality of the medium. The pH control of the medium, and hence of the H^+ concentration, determines the redox level, which permits control of the biological activity towards H_2 production.

4.3.2 Evolution of H_2 Production

Thanks to the acid pretreatment, no methane or hydrogen sulfide production was observed during the process. Only small amounts of water vapour were detected in the gas stream. The H_2 productivity was about 0.90 mol H_2/mol glucose, taking into account the weighted mean of 36 % of H_2 in the total amount of biogas produced (Fig. 4.3). The H_2 productivity obtained in this study, working under uncontrolled ambient temperature and with mixed microflora, is lower than values obtained by many researchers working under mesophilic conditions (35 °C) and with pure fermentative bacteria, such as Clostridia, or with mixed anaerobic culture, such as *C. butyricum* and *Enterobacter aerogenes* [11]. Nevertheless, these results are important in deciding whether the working temperature should be under environmental temperature cycles or not.

4.3.3 The Significance of Tests at Uncontrolled Temperature

The test at uncontrolled temperature demonstrates that it is also possible to produce H_2 under ambient temperature conditions, even under wide fluctuations (24 ± 3 °C). This implies that it could be feasible to operate a full-scale plant with a low energy demand, hence maximizing the net energy production, in contrast to the mesophilic (35 °C) or thermophilic (55 °C) regimes. Regarding this aspect, in the next Chapter we will investigate the temperature effect on bioH_2 production, in order to analyze,

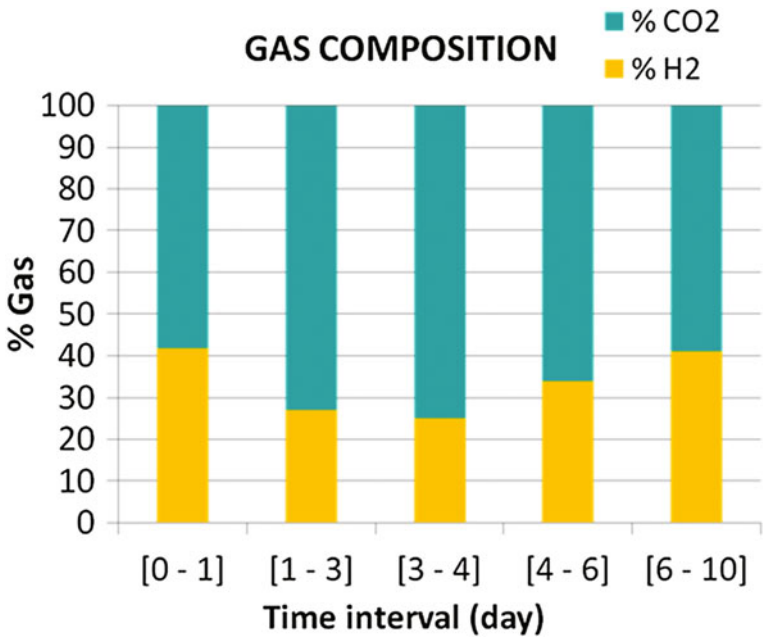

Fig. 4.3 Composition of biogas (CO_2 and H_2) at several time intervals during the test

through a net energy analysis, if it is more convenient to produce a smaller quantity of H_2 with low energy consumption, i.e. working at ambient temperature, rather than to produce a larger quantity of H_2 working at higher temperatures. To this end, we designed and performed the tests reported in the following section.

4.4 Results of Tests at Different Temperatures

4.4.1 Comparison Between Tests at Different Temperatures

Figure 4.4 shows the progress of cumulative hydrogen production in the batch tests at different temperatures. It shows that the duration of H_2 production is very different from one test to another, indicating a strict relationship with the temperature. Figure 4.5 is merely an expansion of Fig. 4.4 on the time axis to better see the lag phase of each temperature tested.

Table 4.1 reports the glucose utilization efficiency, the H_2 yield and the total duration of fermentation; the results show that the greatest glucose utilization efficiencies are obtained at working temperatures of 20 and 35 °C, even though at 35 °C the rate of glucose consumption is almost twice as fast as at 20 °C. The lowest value of glucose utilization occurs at 16 °C. This confirms that at low temperatures the electrons produced by glucose oxidation are mainly utilized for

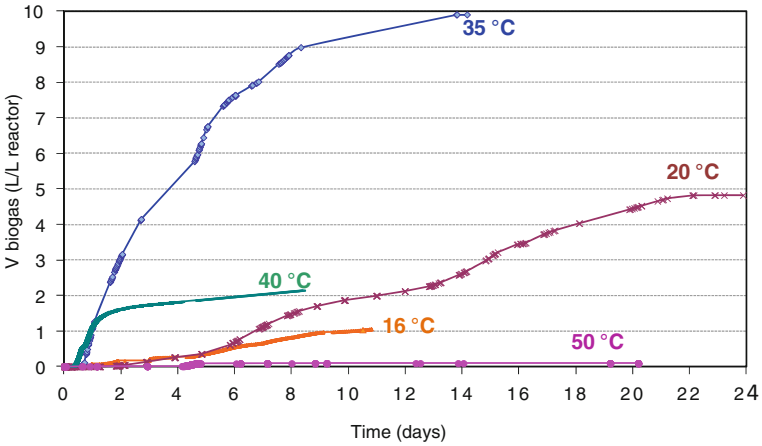

Fig. 4.4 Progress of cumulative bioH_2 production in batch tests at different temperatures

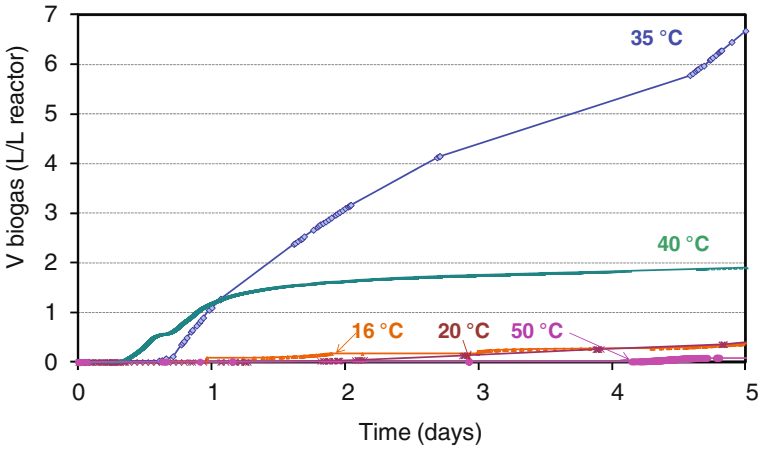

Fig. 4.5 Expansion of lag phase of cumulative H_2 production at different temperatures

Table 4.1 Substrate degradation efficiency and H_2 yield and duration versus temperatures

T_w (°C)	Glucose utilization (%)	H_2 yield (mol H_2/mol glucose)	Δt (days)
16	12.0	0.35	10.8
20	99.6	0.65	23.9
35	99.8	1.21	13.8
40	30.0	0.92	8.4
50	73.0	0.005	12.5

metabolic respiration instead of being used via Fd to reduce protons and produce hydrogen. The maximum Y_{H2} is at 35 °C, followed by the tests at 40 and 20 °C.

Figure 4.6 shows the course of the fermentation test conducted at 50 °C, including the determination of VFA produced. At 50 °C, Y_{H2} is almost zero, but we can suppose that at 50 °C the microorganisms which follow lactic fermentation are dominant in the HPB consortium, considering the large consumption of substrate (73 %) and the great quantity of lactic acid relative to other VFA produced during fermentation, which reach a very high concentration of 23 g/L in about 18 days (Fig. 4.5b).

The strong consumption of sodium hydroxide (2N NaOH) shown in Fig. 4.6 is proportional to glucose consumption; microorganisms oxidize glucose, releasing

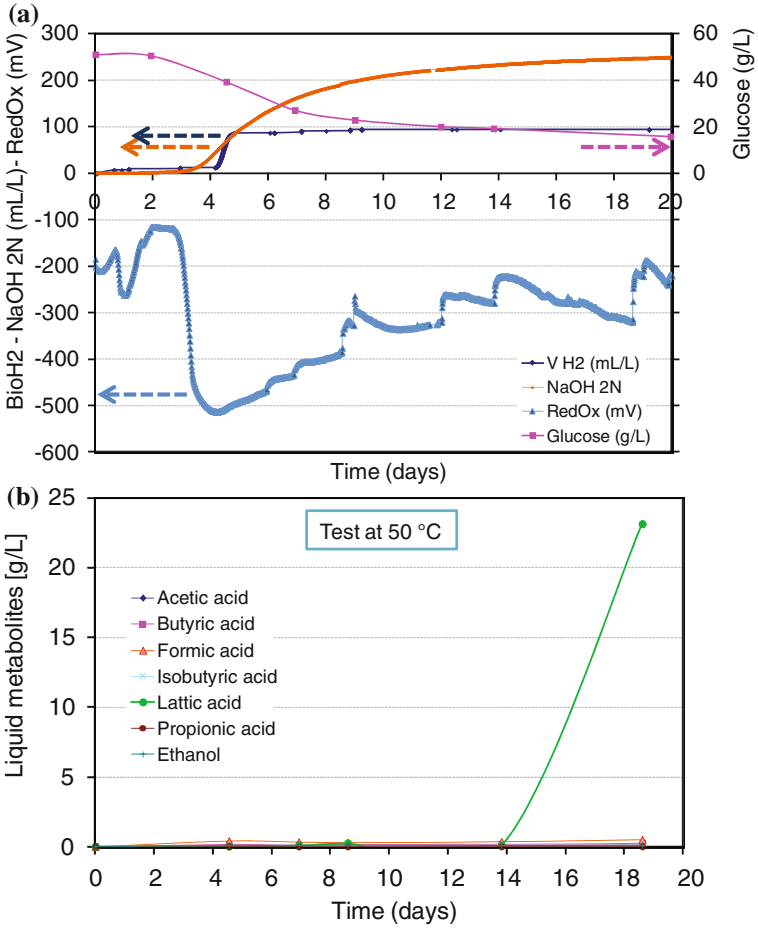

Fig. 4.6 Test at 50 °C. **a** Production of H_2 in relation to redox, pH, glucose consumption and NaOH consumed during fermentation; **b** development of liquid metabolites during fermentation

electrons and protons that create an acidification of the system, which consequently needs NaOH pump intervention to maintain the pH at 5.2. Furthermore, Fig. 4.6a shows the redox trend of the test: it does not remain stable despite constant pH, but moves towards a less reductive range (−300 to −200 mV) after a rapid drop below −550 mV.

Comparison of the redox behaviour in the tests at 20 and 35 °C (see Figs. 4.7 and 4.8, respectively) shows that, when the pH is constant, the redox potential remains at strongly reductive values (about −500 mV) allowing H_2 production until glucose is completely consumed. This confirms the strong role of pH; it decreases correspondingly with H_2 production and with the formation of acidic metabolites expressed by VFA.

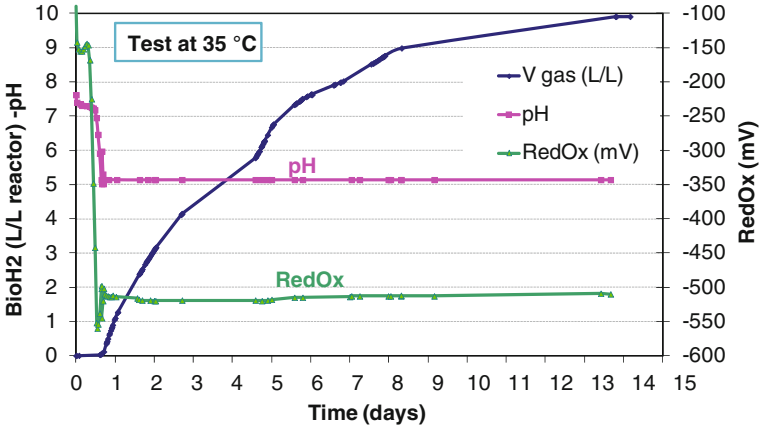

Fig. 4.7 Development of pH, redox and bioH_2 volume in batch test at 35 °C

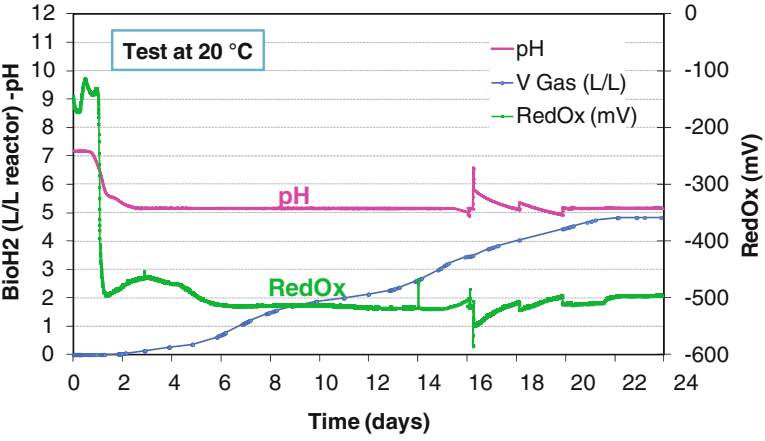

Fig. 4.8 Development of pH, redox and bioH_2 volume in batch test at 20 °C

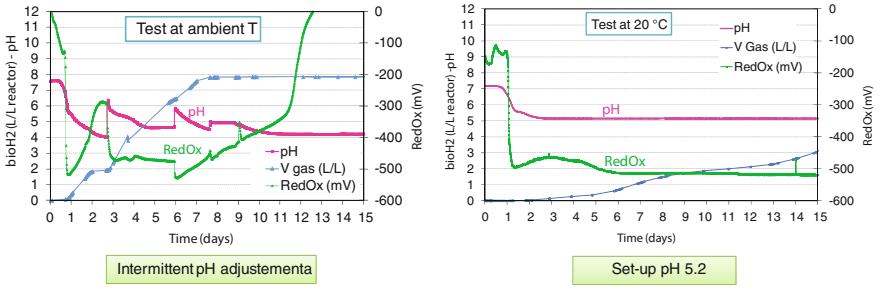

Fig. 4.9 Comparison of tests at ambient temperature and at 20 °C to stress the importance of pH set to 5.2

Figure 4.9 shows the comparison between two tests at approximately the same temperature but with different strategies of pH control: one test at ambient temperature with intermittent pH adjustment and the other one at 20 °C with pH set at 5.2, after the natural decrease to this value. The aim of this comparison is not to compare H_2 yields, since the temperatures are slightly different, but to compare the opposite behaviours of redox depending on pH. If pH is constant (graph on the right) redox is constant and gas continues to develop until the glucose is completely consumed; the opposite occurs in the test without pH control. Recalling that when bacteria grow on organic substrates by oxidation of such substrates the oxidation generates electrons which need to be disposed of in order to maintain electrical neutrality, different compounds, i.e. protons, act as electrons acceptors, which are reduced to molecular hydrogen, maintaining in this way the electron balance. These phenomena can occur only if an adequate difference of electrical potential exists as the driving force, i.e. a reducing environment of neighbouring microorganisms (detected by redox) is necessary to produce H_2.

The increase of redox during the fermentation is due to the production of protons (detected by the rapid drops in pH), in direct correlation to the consumption of substrate. In fact, from the test at ambient temperatures (reported on the left-hand side of Fig. 4.9), we observed that when the pH is very low, the redox is less reduced and biogas shut-down occurs. In contrast, we observe on the right-hand side of Fig. 4.9 that if the pH remains constant during fermentation, i.e. if the medium is well buffered, the protons produced are removed and the redox potential remains constant in a strongly reductive range. As a consequence of constant pH, a greater level of hydrogen production is obtained. From this analysis, we can conclude that it is very important to buffer the pH because it permits a more stable and longer-duration H_2 production, due to the effect of pH on the hydrogenase enzymes that catalyze the reversible reductive formation of hydrogen from protons and electrons:

$$2H^+ + 2e^- \leftrightarrow H_2 \tag{4.1}$$

Adams and Mortenson [12] studied the effect of pH on hydrogenase-catalyzed H_2 evolution. They found that hydrogenases I and II have their maximal efficiency

at pH 6.3 and pH 5.8, respectively, whereas the activity of both enzymes rapidly decreases at pH < 5. The present results confirm the finding of Adams and Mortenson.

4.4.2 Liquid Products from H_2 Fermentation

The soluble metabolites produced by the mixed culture detected in the tests were mainly composed of butyric acid, acetic acid, propionic acid, lactic acid and ethanol.

Table 4.2 shows the percentages present among all soluble metabolites at the end of fermentation: the concentrations of acetic and butyric acid increase from the test at 16 °C to the one at 35 °C, but with a further increase of temperature (40 and 50 °C) they decrease.

In contrast, at 40 and 50 °C high concentrations of lactic acid appear. Considering for both cases the relative small amount of H_2 production, we suppose that a metabolic pathway shift occurred at higher temperatures, induced by different bacteria present in the consortium becoming dominant under less favorable conditions for HPB. The production of H_2 is mainly coupled with acetate and butyrate fermentation pathways, and the anaerobic spore-forming bacteria, Clostridia, form an important part of the acidogenic population performing these fermentation pathways. The production of acetate and butyrate, respectively, from glucose under anaerobic conditions can be explained by the following stoichiometric chemical equations:

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \quad (4.2)$$

$$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2. \quad (4.3)$$

In contrast, the production of propionate requires a consumption of H_2:

$$C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O. \quad (4.4)$$

Table 4.2 Volatile fatty acids and ethanol expressed as percentage of total metabolites at the end of H_2 production versus temperature

T_w (°C)	H_2 produced (mmol H_2/L)	Δt (days)	Acetic acid (% w/w)	Butyric acid (% w/w)	Formic acid (% w/w)	Lactic acid (% w/w)	Propionic acid (% w/w)	Ethanol (% w/w)
16	15.4	6.6	1.22	60.22	1.52	17.98	0.42	18.40
20	215.3	23.9	2.91	81.08	2.67	0.04	0	13.30
35	442.0	13.8	7.93	90.81	0.58	0	0.12	0.56
40	96.0	10.4	5.85	61.2	3.42	15.23	0.9	3
50	1.1	12.5	1.66	3.95	3.05	90.44	0.01	0.90

Thus, in practice, high hydrogen yields are associated with a mixture of acetate and butyrate as fermentation products [13]. In addition, formic acid adds to hydrogen production via its decomposition catalyzed by the hydrogenase enzyme:

$$HCOOH \rightarrow CO_2 + H_2. \tag{4.5}$$

Facultative bacteria like *Enterobacter* spp., *Escherichia coli, Salmonella, Shigella* and *Klebsiella* are responsible for mixed acid fermentation, where the products are the three acids (lactate, acetate and formate) as well as ethanol and equal amounts of H_2 and CO_2; this is also characteristic for members of the Enterobacteriaceae family [14].

In addition, ethanol fermentation leads to H_2 production, as reported by Hwang et al. [15], who conducted ethanol and butyrate–acetate fermentation simultaneously:

$$C_6H_{12}O_6 + H_2O \rightarrow C_2H_5OH + CH_3COOH + 2CO_2 + 2H_2. \tag{4.6}$$

The wide range of options for metabolic pathways is due to the mixed bacterial populations present in mixed consortia, which leads the fermentation towards the most appropriate metabolic pathway for the environmental conditions. Figures 4.10 and 4.11 show the production of liquid metabolites during H_2 production in batch tests at 20 and 35 °C, respectively. Furthermore, in the same graphs we inserted a cumulative H_2 production curve in order to see a correlation with metabolite behaviour during acetogenic fermentation. In the test at 20 °C (Fig. 4.6), butyric acid is distinctly dominant over all the other metabolites, reaching a concentration of 27.43 g/L at 20 days.

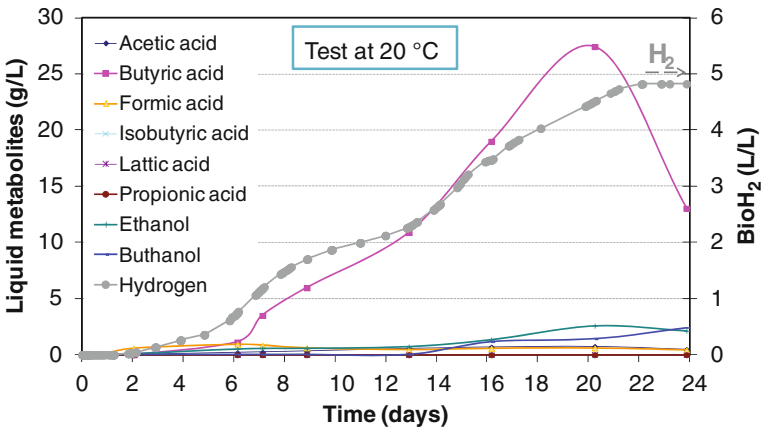

Fig. 4.10 Production of liquid metabolites and hydrogen during batch test at 20 °C

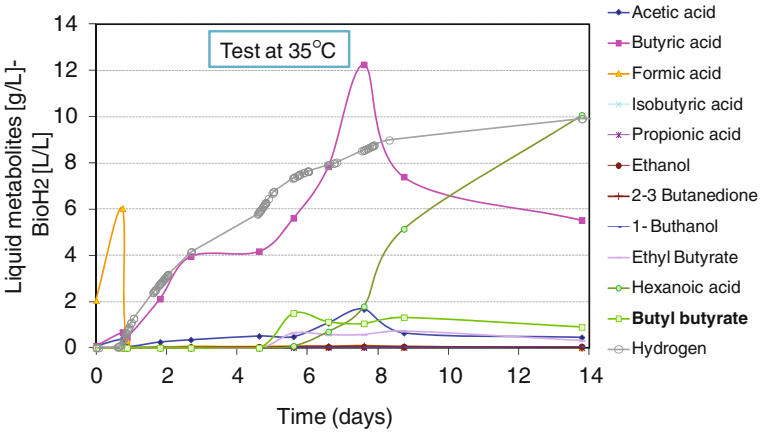

Fig. 4.11 Production of liquid metabolites and hydrogen during batch test at 35 °C

At 35 °C (Fig. 4.11) butyric acid is also dominant (12.42 g/L at 7.6 days), but among all prominent metabolites it is the presence of hexanoic acid ($C_6H_{12}O_2$), also called caproic acid, that becomes more and more evident after about 8 days of fermentation, which coincides with the total consumption of glucose. Probably, microorganisms that follow butyric-type fermentation when glucose is completely consumed utilize butyrate, which in fact decreases, to biosynthesize this saturated acid. At high temperatures (40 and 50 °C) significant amounts of lactate were produced, which becomes the major product at the expense of acetate and butyrate. A marked variation in liquid metabolite production indicates a shift in the metabolic pathways of acid fermentation in the digestion process. Because the metabolic pathway relates to the bioactivity of the dominant microorganisms, the experimental results could be viewed as dependent on the temperature of the different biomass activities.

Figures 4.12a–c show the effect of temperature on H_2 production, H_2 production rate and duration of lag phase, respectively. The results show that the hydrogen production potential in batch tests increased as the temperature increased from 16 to 35 °C, whereas it decreased with further temperature increases from 40 to 50 °C. Among all the tests, the maximum H_2 production and maximum H_2 production rate of almost 10 L H_2/$L_{reactor}$ and 1.5 L H_2 L^{-1} day^{-1} were obtained at 35 °C. At 40 °C the rate of H_2 production was also high, but the fermentation reached a stationary phase early on, although a large quantity of unused glucose remained.

Figure 4.12c shows that lag phase is shorter at 35 and 40 °C than at other temperatures. The shortest lag phase of 9 h was obtained at 40 °C; the temperatures in the range of 30–40 °C probably facilitate the germination and consequently the acclimatization of *Clostridium* spores. For instance, Gibbs [16] found that, for *C. bifermentans,* the optimal temperature between 12 and 45 °C was 37 °C. At temperatures below 20 °C, little or no germination occurred in 60 min. At 45 °C, germination was markedly decreased. According to similar studies [1, 17],

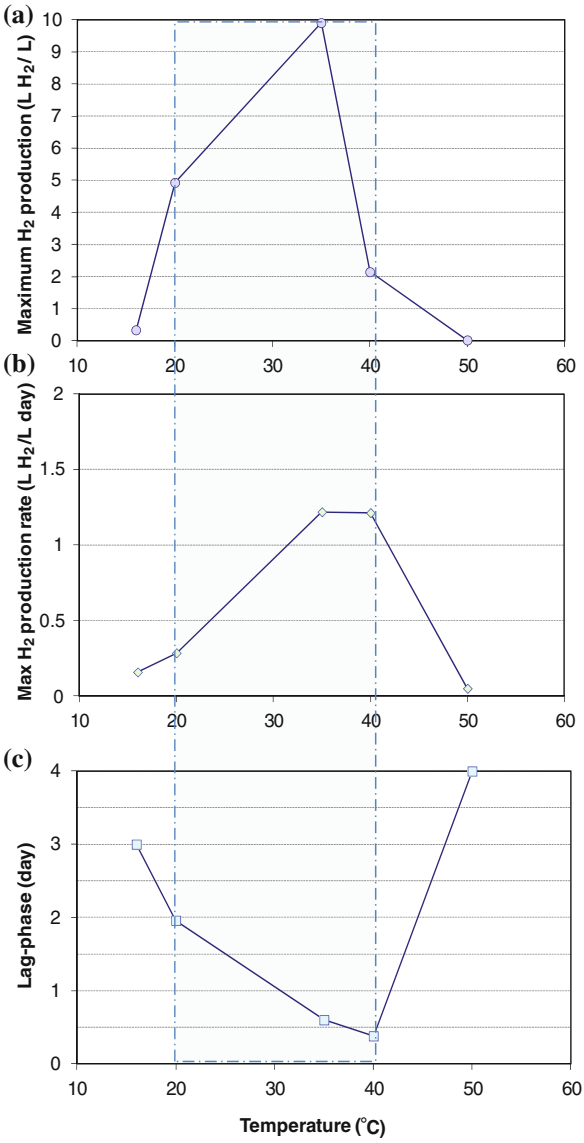

Fig. 4.12 Effect of temperature on maximum H_2 production rate (**a**), maximum H_2 production (**b**) and duration of lag phase (**c**)

an appropriate temperature range can enhance the ability of mixed cultures to degrade substrate; with temperatures increasing from 20 to 35–37 °C the H_2 production and the production rate increase, with further increase in the range 40–50 °C. As a holistic consideration, we can argue that in an appropriate range, between 28 and 40 °C, the temperature can raise the hydrogen production and the maximum hydrogen production rate and can shorten the lag phase of acetogenesis fermentation for H_2 production by mixed cultures. Lastly, we can argue that a

bioreactor producing H_2 could work at a temperature in the range 20–40 °C from a microbiological and industrial point of view. From an engineering point of view, the suggestion is to work at the minimum working temperature, for example at 22–23 °C, in order to maximize the net energy production. In the meantime we suggest to adequately insulate the reactor and to have a temperature control loop with a set point (22 °C) able to use the energy provided free by environmental temperatures during the appropriate seasons.

4.4.3 *Yield of Tests According to Temperatures and Metabolic Products*

As previously seen, the products of acetogenic fermentation (including H_2 and liquid metabolites) vary according to the temperature (Table 4.2), which creates growth conditions better for some species which became dominant to the detriment of others.

The theoretical H_2 yield (Y^*) of the metabolites present in the broth for each working temperature can be calculated by applying Eq. (3.16); the results are shown in Fig. 4.13. The Y^* is far from the theoretical H_2 yield if only acetic acid is present in the broth (4 mol_{H2}/mol_{Glu}). In fact, among liquid metabolites, butyric acid is often the most abundant, and a butyrate fermentation yield of 2 mol_{H2}/mol_{Glu} is the highest value. From Fig. 4.13 the yield is always less than 2, apart from the value at 35 °C that exceeds this threshold. This is also in agreement with several studies by other authors [17].

It is interesting to compare the experimentally evaluated yields of tests at different temperatures with the theoretical ones (Table 4.1); the results are shown in Fig. 4.13. This aspect has a strongly counterface from the energetic point of view. Assuming 2,882 and 240 kJ/mol of lower heating value (LHV) for glucose and

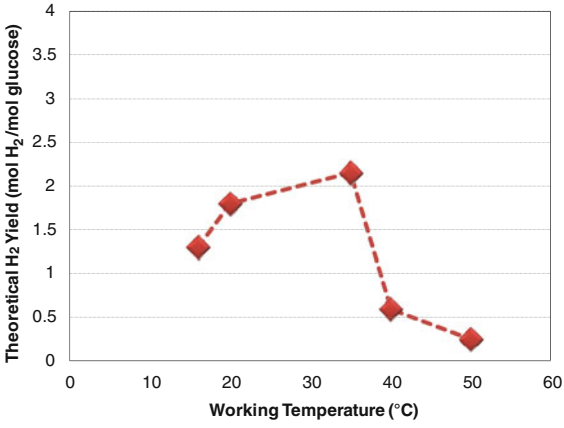

Fig. 4.13 Theoretical H_2 yield according to working temperature

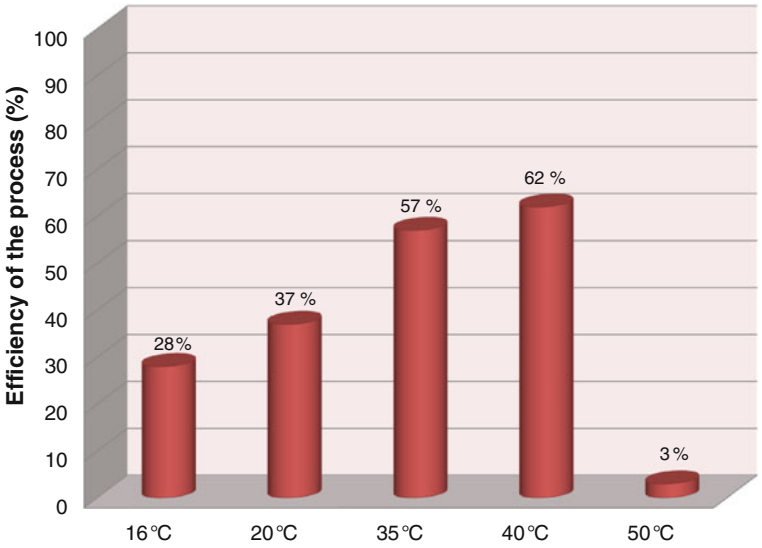

Fig. 4.14 Efficiency of tests conducted at each working temperature

hydrogen, respectively, energy conversion efficiencies of about 33 and 17 % are calculated if acetate or butyrate fermentation pathways are assumed to occur (Eqs. 4.1 and 4.2). Considering the theoretical H_2 yield of Fig. 4.13, the energy conversion at 35 °C is equal to ~18 %, which drops to approximately 11 % taking into account the efficiency from Fig. 4.14.

4.5 Conclusion

In this Chapter the feasibility of converting glucose into hydrogen via dark fermentation in a bioreactor is shown, even at low temperatures such as 20 °C and at ambient temperatures exposed to natural fluctuations. However, the optimal temperature for fermentative H_2 production by mixed cultures is 35 °C. At extreme temperatures, i.e. 16 and 50 °C, the H_2 production is very low, although at 16 °C a residual HPB activity was seen. The variation of H_2 production at different temperatures could be linked to different metabolic pathways as a consequence of the activity of different bacteria species present in a mixed culture of HPB. Furthermore, the buffering of pH is very important for H_2 production, because it permits a more stable and longer-duration H_2 production, even though the cost of NaOH needs to be considered. These indications are very valuable for scale-up purposes.

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