Chapter 6
Hydrogen Production from Biowaste

In this Chapter the feasibility of hydrogen production from organic waste (OW) is highlighted. Possible sources are the residue of municipal solid waste (MSW) sorting by mechanical/physical treatment, the OW separately collected from households and the waste produced along the entire food production chain. A brief review introduces the different kind of utilizable biomass and pretreatment processes necessary to make complex organic substrates easy to be metabolized by HPB. Some experimental tests are then presented with the aim of investigating the feasibility of using OW for H_2 production, selecting an easy and efficacious pretreatment method; tests with fruit and vegetable market wastes were conducted emphasizing the problems in scaling up the technology.

6.1 Biomass as Food for Microorganisms

Biomass in its traditional sense can be defined as "recent organic matter originally derived from plants as a result of the photosynthetic conversion process, or from animals, and which is destined to be utilized as a store of chemical energy to provide heat, electricity, or transport fuels" [1]. The chemical energy contained in the biomass is derived from solar energy by photosynthesis, the process by which plants take carbon dioxide and water from their surroundings and, using energy from sunlight, convert them into sugars, starches, cellulose, lignin etc. Biomass resources include wood from sustainable plantation forests or forest maintenance, residues from agricultural and breeding for food production as well as residues along the alimentary chain, including the production and the distribution, as well as at the points of utilization such as markets. In general, *biowaste* belongs to one of the three following groups [2]:

- animal waste (feces/manures)
- plant materials (grass clippings and vegetable peelings)
- processed materials (food industry and abattoir wastes)

B. Ruggeri et al., *BioH₂ & BioCH₄ Through Anaerobic Digestion*,
Green Energy and Technology, DOI 10.1007/978-1-4471-6431-9_6

OW is a major component in daily MSW and it can comprise more than 50 % if the "paper and card" plus "biowaste" categories are taken into account, as shown in Table 6.1.

Biowaste derived from plant material is principally composed of cellulose, though with different amounts of other plant structural compounds, including hemicelluloses and lignin [3].

Bearing in mind that MSW is composed largely of organic materials, the fermentation process seems to be adequate for both the organic part of MSW and the residues of the agro-industrial sector. Among the organic materials, sugars can be easily fermented into fuels such as ethanol, methane or hydrogen, but most organic materials are polymers with five or six carbon sugar units such as cellulose, hemicellulose and lignin, with different degrees of oxidation. Most of these carbohydrates can be converted into fuels, i.e. ethanol, hydrogen or methane, via biotechnology processes. In order to transform large quantities of organic waste materials into energy vectors, it is necessary to convert the carbohydrate polymers into low-molecular-weight monomer sugars. Sugars, starches, lipids and proteins present in organic waste are those most easily degradable by microorganisms, while

Table 6.1 Typical composition of municipal solid waste in industrialized countries

Category	% (w/w)[a]	% (w/w)[b]	% (w/w)[c]	% (w/w)[d]	Inclusions
Paper and card	33.2	30.7	21.5	32.7	Newspapers, magazines, other papers, liquid containers, packaging card and other card
Biowaste	27	33.7	34.3	25.3	Garden waste, kitchen waste, other putrescibles and fine waste (<10 mm)
Glass	9.3	7.9	10.6	5.3	Brown, green, clear and other glass
Miscellaneous combustibles	8.1	5.2	6.7	5.6	Disposable nappies and other combustibles
Dense plastics	5.9	3.4	6.9	12.1	Clear and colored beverage bottles, other bottles, food packaging and other dense plastics
Ferrous metal	5.7	7.5	3.8	8.2	Beverage cans, food cans, other cans, batteries and other ferrous metals
Plastic film	5.3	4.6	n.a.	7.6	Refuse sacks and other plastic film
Textiles	2.1	3.3	2.6	n.a.	Textiles
Miscellaneous non-combustibles	1.8	2.5	12.6	n.a.	Miscellaneous non-combustibles
Non-ferrous metals	1.6	1.2	1.0	3.2	Non-ferrous beverage cans and other non-ferrous metals

n.a. not available, *w/w* weight/weight

[a] Warren Spring Laboratory data, presented to 1994 Harwell Waste Management Symposium

[b] An introduction to Household Waste Management, ETSU for the DTI, March 1998

[c] Burnley et al. 2007

[d] Centre for Sustainable System, University of Michigan, 2009; http://css.snre.umich.edu

some other fractions, such as lignocelluloses and keratin, are more difficult to degrade. Several enzymes, such as amylase, cellulase, protease, keratinase and lipase, carry out the biological degradation of these polymers, before further fermentation or digestion to produce ethanol or biogas.

Cellulose itself is a structural carbohydrate made up of a complex chain of hexose units. While hemicelluloses superficially resemble cellulose, they are more readily hydrolyzed; lignin however, found in woody tissue, is the most complex of all plant structural carbohydrates and, though originally derived from the conversion of cellulose, it is much less readily decomposed. OW routinely has high water content, often 80–90 % w/w [2], hence biomass has a heat value that can be partly "captured" in other, more immediately useful forms of energy [3], but the high water content makes it a good substrate for biological processes, e.g. anaerobic hydrogen production as well as biogas or bioethanol production. The estimated annual biosynthesis production of biomass is about 170×10^9 t; 75 % is carbohydrate, mainly in the form of cellulose, starch and saccharose, 20 % is lignin and only 5 % is made up of other natural compounds, such as fats (oils), proteins and various substances [4]. It is evident that the main scientific attention towards searching for new organic feedstock should first be focused on efficient use and after-use recovery of carbohydrates. In fact, taking into account that each person produces on average more than 450 kg of MSW per year with about 60 % organic nature, and additional organic waste comes from food chain production and manufacturing, the great necessity of using these materials as feed to produce fuels is evident.

To make most complex and under-utilized organic sources quickly available to microorganisms, pretreatments are necessary. The pretreatment process, by either physical, chemical or biological means, is a well investigated process for ethanol production using lignocellulosic materials. Furthermore, some efforts have been made to treat waste materials in order to increase biogas production. A pretreatment process can enhance the bio-digestibility of the wastes for ethanol and biogas production and the accessibility of the enzymes to the materials, improving the yield of the bioreaction. From the process management point of view, this means that pretreatments are useful to accelerate the rate-limiting step of anaerobic digestion, since bacterial action proceeds more slowly in the hydrolysis stage than in either of the following acidogenic or methanation steps. During hydrolysis, complex insoluble organic polymers, such as carbohydrates, cellulose, proteins and fats, are broken down and liquefied by the extracellular enzymes produced by hydrolytic bacteria. This makes them more easily available for use by acidogenic bacteria.

6.2 Lignocelluloses in Organic Waste Materials

The main structural component of organic waste is cellulose, a biopolymer that consists of thousands of glucose units. Due to the compositional variability of OW, different types of cellulose are present, each of which has its own degree of biodegradability. The cellulose units are linked by β-1, 4-glucoside bonds, and the resulting biopolymers are connected by means of hydrogen bonds of different strengths, which generate different types of cellulose. For this reason, cellulose exhibits different structural features, such as crystalline and amorphous parts. In addition, hemicellulose, a xylose-based biopolymer, offers structural support to these organic materials. Lignin can be found in the woody tissue of plants and provides structural support to the cell wall, as well as impermeability and resistance against oxidative stress and attacks by microorganisms [5]. The structure of hemicellulose looks like that of cellulose and it is easily hydrolysable. Conversely, lignin, although it is derived from the oxidation of cellulose, is an insoluble polymer difficult to degrade because it is composed of a longer chain of complex carbohydrates of high molecular weight than other plant structures. For this reason, in a bioprocess, lignin should be separated from the remaining components by means of an appropriate pretreatment in order to facilitate the transformation of the other structures. Lignocellulosic materials have varying proportions of these three components, usually 40–60 % cellulose, 20–40 % hemicellulose and 10–25 % lignin (see Table 6.2), while the salts are generally less than 10 % of the dry weight of the biomass.

Cellulose has already been described as the most abundant source of food, fuel, and chemicals and its usefulness is due to its ability to be hydrolyzed to glucose. The degradation of cellulose to glucose, also referred to as saccharification, can be accomplished by a chemical reaction (hydrolysis) that involves the addition of a water molecule. Two main methods, enzyme hydrolysis and acid hydrolysis, can affect the degradation of cellulose. Many fungi and bacteria secrete a multicomponent enzyme system called cellulase that has the ability to saccharify cellulose.

Table 6.2 Contents of cellulose, hemicellulose and lignin in common organic waste

Lignocellulosic materials	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Hardwood stems	40–55	24–40	18–25
Softwood stems	45–50	25–35	35–35
Nut shells	25–30	25–30	30–40
Corn cobs	45	35	15
Grasses	25–40	35–40	10–30
Paper	85–99	0	0–15
Wheat straw	30	50	15
Sorted refuse	60	20	20
Leaves	15–20	80–85	0
Cotton seed hairs	80–95	5–20	0
Newspaper	40–55	25–40	18–30
Waste paper from chemical pulps	60–70	10–20	5–10
Primary wastewater solids	5–15	n.a.	24–29
Swine waste	6.0	28	n.a.
Solid cattle manure	1.6–4.7	1.4–3.3	2.7–5.7
Coastal Bermuda grass	25	35.7	6.4
Switch grass	45	31.4	12.0

n.a. not available

Cellulase is composed a pool of four enzymes [5], such as *endoglucanase, celludextrinases, cellubiohydrolases,* and *β-glycosidase*, which act, through a series of reactions, by attacking the accessible cellulose sites till the simplest sugar, glucose, is produced.

The purpose of a pretreatment is to change the intrinsic properties of cellulose in order to prepare the materials for enzymatic degradation, a process which converts the carbohydrate polymers into fermentable sugars. The goal is to break the lignin seal and to disrupt the crystalline structure of cellulose. The best method and operative pretreatment conditions depend to a great extent on the type of lignocellulosic material. The crystallinity of cellulose, its accessible surface area, the degree of cellulose polymerization and the degree of acetylation of hemicelluloses are recognized to be the main factors that are able to affect the rate of biological degradation of lignocelluloses by enzymes. These factors are discussed briefly below. Native cellulose takes on an enzyme-resistant crystalline structure. Fan et al. [6] estimated that the crystalline portion is 50–90 % of the total cellulose, with the remainder being amorphous. It has been shown that cellulase hydrolyzes the accessible portion of amorphous cellulose more easily, while the enzyme is not effective in degrading the less accessible crystalline form. It is therefore widely accepted that reducing the crystallinity increases the digestibility of lignocelluloses. On the other hand, there are some studies [7] that have shown an increased digestibility of most crystalline lignocellulose types. This contrast may appear if the effects of other factors are ignored, for example the role of the pore size, which affects the surface available for the enzyme attack. Fan et al. [6], studying the effect of ball milling, observed an increase in crystallinity with a reduced size of cellulose due to milling. It is believed that re-crystallization during water swelling may increase the crystallinity of highly ball-milled cellulose and consequently the crystallinity might increase the available surface area, probably due to the decrease in pore size. Crystallinity is an important factor for biodegradation of lignocelluloses. However, it is not the only factor that affects enzymatic hydrolysis, because of the heterogeneous nature of celluloses and the contribution of other components, such as lignin. Another important aspect is the accessible surface area. The enzymatic hydrolysis of cellulose is a heterogeneous catalytic reaction, in a chemical reaction sense, and direct contact of cellulolytic enzyme molecules and cellulose is a prerequisite for the reaction. It consists of the adsorption of enzymes from the liquid phases on the surface, the biodegradation of cellulose to simple sugars (mainly cellobiose and oligomers) and desorption of enzymes to the liquid phase. The pore volume of the material determines the contact surface between the cellulose and the enzymes. An increase in the former increases the mass transfer rate, which in turns increases the enzymatic reaction, as several studies have shown [8].

Lignocellulosic materials have two different types of surface area: external and internal. The former is related to the size and shape of the particles, while the latter depends on the pore structure of the cellulosic fibres. Dry cellulosic fibres are small, about 15–40 μm, and they therefore have a considerable external specific surface area, e.g. 0.6–1.6 m^2/g. The internal surface area of dried cellulosic fibres is smaller than the external one. This is due to the hydrogen bond and electrostatic interaction that stabilizes the structures, which tend to reduce the available surface. One operation able to increase the available internal surface consists of lowering the strength of the interactions, by swelling the lignocellulose material with water and/or polar solvents in order to create a larger internal surface area. Drying the fibres could instead produce an irreversible collapse with a reduction in the accessible surface area due to the shrinking of the capillary pores.

In addition, the presence of lignin hemicelluloses and their relative abundance are other important parameters which can affect the pretreatment efficacy. Lignin is the most recognized factor in the recalcitrance of lignocellulosic materials to enzymatic degradation, since cellulose and hemicellulose are cemented together by lignin and this limits the enzyme accessibility (Fig. 6.1).

Several researchers [9] have reported a positive correlation between digestibility/fermentability and the extent of delignification. A correlation level of 40–50 % of delignification is able to increase the digestibility to 90 %, even though some contrasting results have been reported in the literature. Nowadays, it is generally accepted that delignification processes can improve the rate and extent of enzymatic hydrolysis, even though it is difficult to quantify this effect. However, part of the hemicellulose is also hydrolyzed during delignification methods, and so the delignification does not show only one effect. In other cases, it is important to note that the dissolution of the lignin is also an inhibitor of cellulase activity, while less inhibitory effects have been reported concerning xylanase and glucosidase activities [9]. The type of lignin is also significant. It is well known that softwood is more recalcitrant than hardwood and this effect is probably related to the type of lignin, since softwood mainly has guaiacyl lignin while hardwood has a mix of guaiacyl and syringyl lignin. Similarly to lignin, hemicellulose constitutes a physical barrier that surrounds the cellulose fibres and protects them from enzymatic attack. Many pretreatment methods have been shown to be able to remove hemicelluloses, for example the use of dilute sulfuric acid to improve the enzymatic hydrolysis. However, most of these processes act by also removing a part of the lignin, and therefore the improvement is not only due to the removal of hemicellulose.

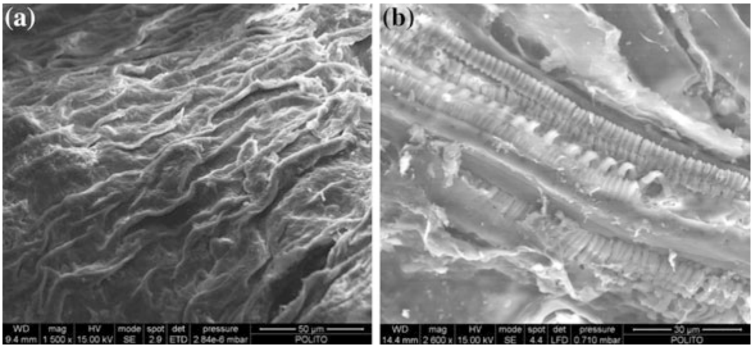

Fig. 6.1 Scanning electron micrography: lignocellulose structure **a** before pretreatment and **b** after pretreatment

6.3 Biomass Pretreatments

Various pretreatment methods are candidates to favor hydrolysis, like milling, irradiation, microwave, steam explosion, ammonia fiber explosion (AFEX), supercritical CO_2 extraction and its explosion, alkaline hydrolysis, liquid hot-water pretreatment, organosolv processes, wet oxidation, ozonolysis, dilute and concentrated acid hydrolysis, and biological pretreatments [10]. The pretreatment can enhance the biodigestibility of the wastes for biogas and bioH_2 production, increasing the accessibility of the enzymes to the biowaste, but the choice of method depends greatly on the type of biowaste. For instance, lignin is the most recalcitrant component of the plant cell wall due to its high resistance to chemical and enzymatic attack [10], and it is a very complex molecule constructed of phenylpropane units linked in a three-dimensional structure which is particularly difficult to biodegrade. Technological factors such as energy demand should be carefully considered when selecting the pretreatment process according to the biomass, bearing in mind that some methods are efficacious for some biomasses but not for some others. A good criterion for feed selection in order to have profitable bioH_2 production is the right balance of the following points:

- biomass availability near the plant, to avoid excessive energy transport costs;
- easy biodegradability of biomass, in order to avoid high costs for the pretreatments;
- biomass from disposal problems is preferable to dedicated plantations;
- fruit and vegetable biomass refuse is preferable in order to overcome food–fuel conflict;
- complicated and high-energy-demand pretreatment methods that are rarely applied at full scales, like microwave, electron-beam and gamma-ray irradiation, vibro-energy milling and ozonolysis, should be avoided.

On the other hand, an effective and economical pretreatment should meet the following requirements:

- minimize the energy demand by reducing the cost of construction materials for pretreatment devices;
- avoid formation of possible inhibitors of hydrolytic enzymes and fermenting microorganisms.

Thus, after the above considerations, it is possible to conclude that in the OW feedstock material there is a large fraction of biomass of difficult digestibility, hence it is important to focus precisely on the effect of the pretreatment; only some pretreatments, among the interesting ones for full-scale application, will be considered here, but additional details can be found in [11]. It is important to remark that some of them are successfully applied to different waste streams originating from various agro-industries, agriculture and municipalities, but to date they remain in the infancy stage from a technological point of view.

Pretreatment methods are classified into *physical, physical–chemical, chemical* and *biological;* the following sections provide a brief description of each of them.

6.3.1 Physical and Physical–Chemical Pretreatments

Physical pretreatment normally involves a mechanical size reduction step which increases the accessible surface area to hydrolytic bacteria, and reduces the crystallinity and the degrees of polymerization of complex substrates prior to further pretreatments. Different types of physical processes such as *mechanical comminution* (e.g. ball milling, two-roll milling, compression milling and vibro-energy milling), *irradiation* (e.g. by gamma rays, electron beam, ultrasound and microwaves), *steam explosion* and *hydrothermolysis* can be used to improve the enzymatic hydrolysis and biodegradability of biowaste. These mechanical pretreatment techniques are time-consuming, energy-consuming and expensive for the process. Compression milling and steam explosion are apparently the only processes that have been tested at production scale. On the other hand, the irradiation techniques and hydrothermolysis have difficulties in industrial application and the processes' economy is still unclear [10, 11]. Therefore, in this context, we will exclude their description and will present only mechanical comminution and steam explosion pretreatments.

6.3.1.1 Mechanical Comminution

Milling is a necessary first step in pretreatment in order to make the subsequent operations more efficacious. Among milling processes, the triturator, colloid mill, fibrillator, ball mill and dissolver are suitable for wet materials. A reduction in the size of organic waste is obtained through a mechanical pretreatment that increases the amount of soluble organic material. Shredding, pulping, crushing, or otherwise reducing the size of the waste gives bacteria access to a greater surface area. For example, a hydropulper produces two fractions, heavy and light, from incoming OW, but it also creates mixed organic slurry. The pretreatment technologies are different as far as the distribution of the chemical components in the waste between the fermentable fraction and rejected material is concerned, especially for dry matter such as ash, plastic or paper. When the source-separated organic waste is pressed through narrow slits, the screw presses the organic matter to form an organic fraction, while items such as plastic, paper, wooden substances, animal bones and metal are routed through the chamber into the reject fraction. Before treatment in the screw press, disk screen or extruder, the collected waste is treated with a "bag-opener", which consists of large slowly rotating coils that cut open any bags and mix the waste. A pretreatment with a shredder (size reduction) and magnet separator to remove the metals requires a waste fraction without any impurities, for example plastic. This means that it is necessary to pretreat the MSW before the pretreatment process that is specifically dedicated to rupturing the lignocellulose structure. Waste materials can be comminuted by a combination of chipping, grinding and milling to reduce crystalline cellulose, and the size of the materials is usually 10–30 mm after

Table 6.3 Energy requirement for mechanical comminution of agricultural lignocellulosic materials with different size reduction

Lignocellulosic materials	Final size (mm)	Energy consumption (kWh/tonne)	
		Knife mill	Hammer mill
Hardwood	1.60	130	130
	2.54	80	120
	3.2	50	115
	6.35	25	95
Straw	1.60	7.5	42
	2.54	6.4	29
Corn stover	1.60	NA	14
	3.20	20	9.6
	6.35	15	NA[b]
	9.5	3.2	NA[b]

chipping and 0.2–2 mm after milling or grinding [12]. The power requirement for mechanical comminution of agricultural materials depends on the final particle size and the waste biomass characteristics [5]. A comparison is shown in Table 6.3.

6.3.1.2 Steam Explosion

The steam explosion pretreatment method for lignocellulosic materials is conducted in the presence or absence of acid or basic chemicals in wet or dry conditions. In this method, a mechanically treated biomass is processed with high-pressure saturated steam for a certain time and then the pressure is swiftly reduced, which makes the materials undergo an explosive decompression. Steam explosion is usually conducted at a temperature of 160–260 °C, with a corresponding pressure of 0.69–4.83 MPa, for a period of time that ranges from several seconds to a few minutes, before the material is exposed to atmospheric pressure. The process causes hemicellulose degradation and lignin transformation due to the high temperature, and thus increases the potential of cellulose hydrolysis. Ninety percent efficiency of enzymatic hydrolysis has been achieved in 24 h for poplar chips pretreated by means of steam explosion, compared to only 15 % hydrolysis of untreated chips [13]. The residence time, temperature, size and moisture content affect steam explosion efficiency. The size determines the contact area between the steam and the material of the enhancing mass transfer phenomena, while the moisture affects so-called auto-hydrolysis. The latter phenomenon is of particular importance in processes conducted without acid or base addition, in which the hydrolysis is catalyzed by acids liberated from functional groups such as acetyl. Optimal conditions are defined as those in which the best substrate for hydrolysis is produced with the minimum amount of material being lost due to side reactions, such as dehydration, and in which adequate carbohydrate linkages are disrupted by releasing most of the hemicelluloses into solution while leaving the cellulose fraction intact. Steam explosion is one of a very limited number of pretreatment techniques that have been made available through pilot-scale demonstrations [12], and commercial equipment is currently available, even though its use for OW of MSW is limited. The temperature and the duration determine the chemical changes in the three main constituents of lignocellulosic materials. The hemicelluloses are hydrolyzed to soluble sugars by the organic acids, mainly acetic acid derived from the acetylated hemicelluloses in straw. The pH during steam explosion is kept low (3–4) to degrade a higher quantity of hemicelluloses. However, under more drastic conditions involving higher temperatures or longer exposure times, the solubilized hemicelluloses undergo a series of secondary reactions and form furfural and hydroxymethyl furfural. The addition of H_2SO_4 (or SO_2) or CO_2 can effectively improve enzymatic hydrolysis, decrease the production of inhibitory compounds and lead to a more complete removal of hemicelluloses. One of the advantages of steam explosion pretreatment is the low amount of energy that is required compared to mechanical comminution. Conventional mechanical methods require 70 % more energy than steam explosion to achieve the same size reduction. Steam explosion is recognized as one of the most cost-effective pretreatment processes for hardwood and agricultural residues, but it is less effective for softwood [14]. The limitations of steam explosion include destruction of a portion of the xylan fraction, incomplete disruption of the lignin–carbohydrate matrix and generation of compounds that may be inhibitory to the microorganisms used in downstream processes. Because of the formation of degradation products that are inhibitory to microbial growth, a pretreated biomass needs to be washed with water to remove the inhibitory materials along with water-soluble hemicellulose. A water wash decreases the overall saccharification yields, due to the removal of soluble sugars, such as those generated by hydrolysis of hemicelluloses. Usually, 20–25 % of the initial dry matter is removed by a water wash. Steam explosion and thermal pretreatments are being widely investigated for improving biogas production from different dedicated materials such as forest residuals and wastes such as cattle manure or municipal solid wastes [15]. However, there are several investigations combining thermal pretreatment with addition of bases such as NaOH, which usually gives a better result than individual thermal or chemical ones [16].

6.3.2 *Chemical Pretreatment*

Chemical pretreatment techniques have received by far the most attention among all categories of pretreatment methods. Typical examples include dilute acid, alkali, solvent, ammonia, SO_2, CO_2, other chemicals (e.g. $H_2O_2 + Mn^{2+}$, ozone, EDTA, $KMnO_4$, urea and dioxane) and pH-controlled hydrothermolysis. Of these pretreatments, the alkaline and acid ones have been successfully tested on a pilot scale.

On the other hand, the use of organic solvents (the *organosolv* process) is expensive and their use is complex, requiring high-pressure equipment; ammonia pretreatment [e.g. ammonia fiber/freeze explosion (AFEX) and supercritical ammonia pretreatment] have not been tested on a pilot scale; SO_2 steam explosion seems appealing because it can effectively render softwoods digestible but SO_2 is highly toxic and may pose safety and health challenges [11]; explosion pretreatment in the presence of CO_2, with or without steaming, seems an effective technique [17] although it has not been tested on a pilot scale; other chemicals tested as pretreatment agents are generally ineffective or too expensive and only bench-scale tests have been reported; pH-controlled hydrothermolysis can enhance enzyme digestibility by controlling the pH during pretreatment but has not been fully investigated for process characteristics and process economy [11].

After this short overview, acid and alkaline pretreatments will be considered, since they are the most inexpensive and effective techniques.

6.3.2.1 Acid Pretreatment

Concentrated acids, such as H_2SO_4 and HCl, have been used to treat lignocellulosic materials. Although they are powerful agents for cellulose hydrolysis, concentrated acids are not extensively used due to problems of toxicity and hazard. Acid treatment can operate either under high temperature and low acid concentration (dilute acid treatment) or under low temperature and high acid concentration (concentrated acid treatment) [10]. The lower operating temperature in concentrated acid pretreatment (e.g. 40 °C) is a clear advantage compared to dilute acid processes from an energy point of view. However, high acid concentrations (e.g. 30–70 %) in the concentrated acid process makes it extremely corrosive and dangerous. Therefore, this process requires either specialized non-metallic constructions or expensive alloys. Pretreatments with acids such as acetic, nitric and sulfuric have also been used to remove lignin and cellulose from waste newsprint [18] and active sludge for biogas production. Of all the acid-based pretreatment techniques, sulfuric acid has been the most extensively studied, apparently because it is inexpensive and effective. The potential difficulties of the need for corrosion-resistant construction materials for reactors and gypsum generation, however, plague sulfuric acid's prospects as a long-term pretreatment chemical [11]. Direct saccharification results in low yields at moderate temperatures of around 40 °C with high acid concentration, because of sugar decomposition. High temperatures in dilute acid treatments favor cellulose hydrolysis. A neutralization of pH is necessary for downstream enzymatic hydrolysis or fermentation processes. Most of the arabinan, galactan and xylan in the biomass is hydrolyzed during the acid pretreatment. The cellulose remaining in the pretreated feedstock is highly digestible by cellulases from *Trichoderma reesei* [19]. This pretreatment produced a significant increase in the bioavailability of cellulose to hydrolysis enzymes. A wide range of feedstock materials, including agricultural residues and wastepaper, has been treated with acid.

6.3.2.2 Alkaline Pretreatment

Alkaline pretreatment involves the use of alkaline solutions such as sodium hydroxide (NaOH), lime [$Ca(OH)_2$] or ammonia to remove lignin and a part of the hemicellulose structures to efficiently increase the accessibility of enzymes to cellulose [12]. The effect of an alkaline pretreatment depends on the lignin content of the materials, which would seem to suggest that bases mainly remove lignin. The mechanism of alkaline hydrolysis is the saponification of intermolecular ester bonds cross-linking xylan hemicelluloses and other components, for example lignin and other hemicelluloses. The mechanism of action seems to be due to the increase in porosity of the lignocellulosic materials as a consequence of the saponification reactions. Dilute NaOH treatment of lignocellulosic materials causes swelling and provokes different effects: an increase in the internal surface area, a decrease in the degree of polymerization, a decrease in crystallinity, separation of the structural links between the lignin and the carbohydrates, and disruption of the lignin structure [20]. Pretreatments can be performed at low temperatures but with a relatively long time and a high concentration of the base until pH 12 is reached. Alkaline pretreatment was shown to be more effective on agricultural residues than on wood materials. The digestibility of NaOH-treated hardwood increased from 14 to 55 % with a decrease in the lignin content from 20 to 55 %. In contrast, no effect of dilute NaOH pretreatment was observed for softwood with a lignin content greater than 26 %. The highest yield was obtained under the most severe conditions (alkaline peroxide, 120 °C and 120 min). Furthermore, bases such as $Ca(OH)_2$ could be a good solution when high loads of, e.g., lipids and phenolic compounds are subjected to anaerobic digestion [11]. Olive mill effluent is an example of seasonal waste with low pH (about 4.3) and high concentrations of lipids (about 13 g/L) and phenolic compounds (about 8 g/L). Addition of lime and bentonite greatly improves the digestion of olive mill effluents, with more than 91 % removal of COD [21]. Pretreatment with NaOH at a ratio of 5 g of base/kg of MSW increased biogas production under anaerobic fermentation by 35 % compared with untreated MSW [22].

6.3.3 Biological Pretreatment

This category comprises pretreatment techniques of applying lignin-solubilizing microorganisms to render lignocellulosic materials amenable to enzyme digestion [11].

In biological pretreatment processes, microorganisms such as brown, white and soft-rot fungi are used to degrade lignin and hemicellulose in waste materials. Brown rot fungi mainly attack cellulose, while white and soft rot fungi attack both cellulose and lignin. White rot fungi are the most effective basidiomycetes for biological pretreatment of lignocellulosic materials [20]. The advantages of biological pretreatment include low energy input, no chemical requirement and mild environmental conditions. However, the rate of hydrolysis in most biological pretreatment processes is very low; therefore, biological pretreatments face major techno-economic challenges. Thus far, only bench-scale studies are known to have been conducted.

6.4 Biomass Feedstock for bioH_2 Production: An Overview

Food waste is the major component in MSW, and it accounts for the majority of the research on bioH_2 production [23]. It is composed of raw and cooked food discarded before or during food preparation; it includes high volatile solids, moisture content and salinity, and thus it is the main source of odor, decay, vermin attraction, groundwater contamination and greenhouse gas emission. Food waste has therefore gained interest as a potential feedstock for bioenergy. In the literature, the food wastes employed for bioH_2 studies are obtained mainly from dining-hall or restaurant waste. Food processing wastes such as tomato residue, cheese whey, rice slurry and apple pomace have also been tested for bioH_2 production [23]. In addition, solid wastes such as wheat, starch, the organic fraction of MSW containing fruit and vegetable waste, jackfruit peel etc. can be considered for hydrogen production [24].

BioH_2 production from food waste containing carbohydrates, fats, cellulose and hemicelluloses involves different metabolic pathways, which have not yet been studied in detail [25]. In general, bioH_2 production results from carbohydrate degradation through the acidogenesis and acetogenesis routes and is highly sensitive to certain environmental conditions such as pH, volatile fatty acids, temperature, hydrogen partial pressure, inoculum sources and food waste concentration, as reported in Chap. 2. With increasing energy demand worldwide, utilizing renewable resources such as food wastes and food processing wastes for bioH_2 production can be a novel and promising approach to substituting fossil fuels while at the same time solving disposal problems. A variety of alternative organic biowastes have been effectively used for H_2 production by anaerobic digestion, as listed in Table 6.4.

In particular, Table 6.5 shows literature data on values of H_2 productivity from different biowaste by AD processes, using mixed microbial cultures.

However, in the literature, the different pretreatment methods have shown different degrees of success, and in many cases the best pretreatment in one study is the worst in another one. This discrepancy could be due to the different uses of the substrate and/or the different types of inoculum. To overcome the above difficulties, we suggest scoring the pretreatment methods using two energy parameters, efficiency and efficacy, introduced previously. In the following Sect. 6.5 the results of a study aimed at establishing the proper pretreatment for each type of substrate present in the OW are reported. Different refuses were tested with low, medium or high concentrations of lignocellulosic material to obtain a representative picture of the entire palette of OW. The pretreatment option was evaluated on some food

Table 6.4 Examples of feasible biomass for bioH_2 production

Manufacturing wastes	Food waste	Agro-waste	Other organic waste
Paper sludge	Cafeteria waste	Molasses	Waster activates sludge
Bean curd	Sugar beet juice	Sugary wastewater	Sewage sludge
Cheese whey	Mixed food waste from Restaurant	Vegetable mixed waste	Municipal wastewater
Rice slurry	Fruit juice	Starch wastewater	Municipal solid waste
Apple processing	Citrus peelings	Rice straw	Liquid swine manure
Palm and olive oil mill effluent		Sugar cane bagasse	
Olive mill		Fruit mixed waste	
Rice winery			
Beer lees			

Table 6.5 Yield of bioH_2 production from biowaste by mixed microorganisms

Biowaste	Mode of operation	Mineral or vitamin supplements	pH/Temp	Yield	References
Apple processing wastewater	Batch	With	6.0	0.9 L H_2/L_{medium} (0.1 L H_2/g_{COD})	[26]
Food waste	Continuous	Without	6.5/35 °C	0.39 L H_2/g_{COD}	[27]
Molasses	Continuous	Without	7.0/35 °C	5.57 $m^3H_2/m^3_{reactor}$/day	[28]
Rice slurry	Batch	With	4.5/37 °C	0.35 L $H_2/g_{carbohydrate}$	[29]
Glycerol	Batch	With	6.5/35 °C	0.025 L H_2/g_{COD}	[30]
Beer lees	Batch	With	35 °C	0.053 L $H_2/g_{g\ dry\ beer\ lees}$	[31]
Cheese whey	Continuous (UASB)	Without	5.0/30 °C	0.05 L H_2/g_{gSSV}/day	[32]
Kitchen waste	Continuous (Inclined plug flow reactor)	Without	5.5	0.07 L H_2/g_{gSSV}	[33]
Liquid swine manure	Feed–batch (anaerobic sequencing batch reactor)	With	5.0/37 °C	1.4 mol H_2/mol glucose	[34]
Palm oil mill effluent	Continuous (HRT = 5 d)	Without	5.0	0.42 L H_2/g_{COD}	[35]
Fruit and vegetable waste	Batch	Without	5–6/ 35 °C	3.0 L H_2/L_{medium}	[36]

industrial wastes, using the AD process for bioH_2 production as a probe test to compare the efficiency of different pretreatment options. This analysis aims to provide additional knowledge on the mechanisms of action and to provide useful information at an industrial scale. Three types of refuse were selected: sweet product residue (SPR), organic waste market (OWM), and coffee seed skin (CSS) waste. The SPR, OWM and CSS refuses contain various levels of lignocellulosic material (low, medium and high, respectively) and different amounts of sugar (high, medium and low, respectively). These residues were chosen to understand the feasibility of using refuse from the entire food chain as raw material for hydrogen production. The types of waste from the food industry tested were: SPR, i.e. confectionary residues removed from the market after the expiration date and manually separated from packaging; SPRex, similar to SPR but the packaging was separated by extrusion at 200 atm; OWM residue taken from a local fruit and vegetable market; and CSS residue of a firm producing coffee. Table 6.6 shows the composition of the different types of refuse. The composition of SPR and OWM was defined using the information reported on the packaging and in the INRAN database [37]. The composition of the CCS refuse, which is also reported in Table 6.6, was provided by an indipendent laboratory.

6.5 Experimental Tests from Renewable Agro-Waste

The following sections present experimental tests aiming to produce hydrogen from different OW with different degree of lignocellulose material pretreated by different methods. After preliminary tests conducted in flasks to understand the best pretreatment to hydrolyze the biomass, we performed a test using an anaerobic stirred reactor, controlling physical parameters such as temperature and pH, in order to confirm the efficiency of H_2 production.

6.5.1 Investigation on Pretreatments and H_2 Production

6.5.1.1 Pretreatments of Feedstock

Different pretreatment processes were tested: chemical (acid or basic), thermal (high temperatures for different times), ultrasonic, and a combination of the aforementioned treatments: acid/thermal and basic/thermal. All three refuses were initially mechanically treated with a kitchen blade mixer, which was used to liquidize the material to simulate an industrial milling treatment. A combination of different pretreatments may be optimal to increase the bioreaction yield in many situations. All the pretreatments used are briefly described below and the abbreviations are summarized in Table 6.7. Owing to the large variation in the composition of OWM over time, for this waste, several pretreatment options were tested to identify which one was the most adequate.

Table 6.6 Composition of the residues used

SPR	Parameters	Proteins	Carbohydrates	Sugars	Fat	Saturated fat	Dietary fiber		Sodium
	(g/mL)	0.04	0.29	0.11	0.12	0.06	0.01		3×10^{-4}
	(% w/w)	5.99 %	46.08 %	17.52 %	20.08 %	8.94 %	1.34 %		0.05 %
OWM	Constituents	g	% (w/w)	Water	Soluble sugars	Starch	Proteins	Lipids	Fiber
	Tomato	186	6.7	174.84	6.51	0.00	1.86	0.37	3.72
	Apple	268	9.7	228.34	26.80	0.00	0.54	0.00	6.97
	Orange	280	10.2	244.16	21.84	0.00	1.96	0.56	4.48
	Eggplant	215	7.8	199.31	5.59	0.00	2.37	0.86	5.59
	Lettuce	158	5.7	148.99	3.48	0.00	2.84	0.63	2.37
	Lemon	150	5.4	134.25	3.45	0.00	0.90	0.00	2.85
	Pepper	148	5.4	136.60	6.22	0.00	1.33	0.44	2.81
	Cabbage	161	5.8	148.44	4.03	0.00	3.38	0.16	4.19
	Celery	137	5.0	120.97	3.01	0.27	3.15	0.27	2.19
	Strawberry	156	5.7	141.18	8.27	0.00	1.40	0.62	2.50
	Grape	130	4.7	104.39	20.28	0.00	0.65	0.13	1.95
	Cucumber	156	5.7	150.54	2.81	0.00	1.09	0.78	1.25
	Fennel	180	6.5	167.76	1.80	0.00	2.16	0.00	3.96
	Pumpkin	167	6.1	157.98	5.85	1.50	1.84	0.17	0.00
	Kiwi	205	7.4	173.43	18.45	0.00	2.46	1.23	4.51
	Green bean	60	2.2	54.30	1.44	0.00	1.26	0.06	1.74
	Composition of OWM	2757	100.0	2485.48	139.83	1.77	29.19	6.28	51.08
				90.15 %	5.07 %	0.06 %	1.06 %	0.23 %	1.85 %
CSS	Hemicellulose	Cellulose	Lignin	Humidity	Ethanol-soluble substances			Water-soluble substances	
	(% w/w)	(% w/w)	(% w/w)	(% w/w)	(% w/w)			(% w/w)	
	11.20	31.30	37.30	5.31	5.80			14.07	

Table 6.7 Pretreatments tested, energy produced as H_2 and evaluation of efficiency (η) and efficacy (ξ)

	Biowaste	Nomenclature	Embedded energy (kJ/L)	Produced energy (kJ/L)	η	ξ
SPR	No pretreatment	NP	5320.77	0.99	0.0186	1.0
	Acid pretreatment	AP	5320.77	0.35	0.0066	0.4
	Basic pretreatment	BP	5320.77	6.28	0.1181	6.4
	Thermal acid pretreatment	TAP	5320.77	0.02	0.0005	0.0
	Thermal basic pretreatment	TBP	5320.77	1.39	0.0261	1.4
SPR_{ex}	No pretreatment	NP	1503.70	2.13	0.1408	1.0
	Basic pretreatment	BP	1503.70	24.10	1.6023	11.4
	Thermal basic pretreatment	TBP	1503.70	35.93	2.3897	17.0
OWM	No pretreatment	NP	755.08	2.53	0.3344	1.0
	Acid pretreatment	AP	755.08	17.00	2.2499	6.7
	Basic pretreatment	BP	755.08	4.39	0.5814	1.7
	Thermal pretreatment (10 min)	TP_{10min}	755.08	10.53	1.3941	4.2
	Thermal pretreatment (90 min)	TP_{90min}	755.08	10.97	1.4530	4.3
	Thermal acid pretreatment (15 min)	TAP_{15min}	755.08	23.42	3.1008	9.3
	Thermal basic pretreatment (5 min)	TBP_{5min}	755.08	18.22	2.4134	7.2
	Thermal basic pretreatment (15 min)	TBP_{15min}	755.08	16.08	2.1291	6.4
	Thermal basic pretreatment (30 min)	TBP_{30min}	755.08	25.08	3.3224	9.9
CSS	No pretreatment	NP	755.08	2.27	0.0891	1.0
	Thermal basic pretreatment[a]	TBP_a	2553.13	11.23	0.4399	5.0
	Thermal basic pretreatment[b]	TBP_b	2553.13	10.93	0.4286	4.8
	Ultrasonic pretreatment	UP	2553.13	11.83	0.4640	5.2

[a] Thermal basic pretreatment at 30 °C

[b] Thermal basic pretreatment at 90 °C

Acid pretreatment (*AP*): AP was carried out in a thermostatic chamber at 30 °C. The feedstock was treated for 24 h at pH 3, obtained with the addition of 2 N HCl. This pretreatment, which is also referred to as dilute acid hydrolysis, is one of the most commonly used methods. *Basic pretreatment* (*BP*): BP was carried out in a thermostatic chamber at 30 °C at pH 12 for 24 h using 2 N NaOH. The effect of basic pretreatment appears to suggest that the base mainly removes lignin. *Thermal pretreatment* (*TP*): TP was carried out in a 20 L bench reactor equipped with a controlled mixer and a heating jacket at 90 °C and 2 atm for different time periods ranging from 5 to 90 min; approximately 60 min were necessary to reach the working temperature. Hemicellulose, heated to 150 °C, would already be partially solubilized into constituents, i.e. xylan and glucomannan, the former being less stable, depending on the nature of the substrate. During a thermal process, part of the hemicellulose is hydrolyzed into acids, which are produced together with other chemicals that are able to enhance the hydrolysis of hemicelluloses. *Thermal acid pretreatment* (*TAP*): this pretreatment was carried out in the bench reactor at pH 3 with the addition of 2 N HCl to the broth at 90 °C and 2 atm for 30 min for SPR and 15 min for OWM. The purpose of combining the two processes was to increase the efficiency of the hydrolysis of the lignocellulosic components and to catalyze the solubilization of the hydrolysis products to prevent any inhibition phenomenon. *Thermal basic pretreatment* (*TBP*): TBP was conducted under different conditions for each type of refuse. The pretreatment was carried out at pH 12 for SPR and SPRex, using 2 N NaOH at 90 °C and 2 atm for 30 min. The OWM pretreatment was carried out under the same conditions as SPR, but for different lengths of time: 5, 15 and 30 min. Two different conditions were considered for CSS. The first condition was at pH 12 with 2 N NaOH under gentle heating from 20 to 96 °C at atmospheric pressure. When the temperature reached this value, the heating was stopped, and the broth was left to return to 20 °C; the process lasted approximately 180 min. The second condition for the TBP was pH 12, 90 °C and 2 atm, with the temperature maintained at this value for 30 min. As for the TAP, the aim was to increase the amount of low-molecular-weight compounds and to improve their solubilization. *Ultrasonic pretreatment* (*UP*): UP was carried out in an ultrasonic machine (Ultrasonic Cleaner model CP823) at 30 °C and atmospheric pressure with a power of 1.8 kW for 20 min. In this case, the aim was to verify the capacity of this innovative pretreatment to break down large molecules and to convert them into smaller ones, which are more easily digested by microorganisms.

6.5.1.2 Experimental Tests of H_2 Production

The anaerobic microflora used in these tests were obtained following the procedure reported in Chap. 2, in order to have as much as possible the same quality of inoculum using bovine manure. Tests were carried out in triplicate using 500 mL Erlenmeyer flasks continuously agitated on a shaker at 200 rpm for the entire duration of the test. The mixing rate was selected for two reasons: primarily to favor the evolution of dissolved H_2 in the broth and to prevent inhibition of the bioreactio, n and secondly to increase the shear-stress on HCB to decrease their vital activity. The working volume of each test was approximately 333 mL, comprising 300 mL of broth and 33 mL of inoculum. The initial pH of the broth was in the range 7–7.5 and was adjusted by 2 N NaOH. The flasks were initially flushed with nitrogen gas for 5 min to obtain anaerobic conditions. The treated sludge was used as inoculum in a ratio of 10 % v/v. All the experiments were conducted at 30 ± 1 °C in a thermostatic chamber without control. Gas composition was evaluated at the end of each test as average composition. The measures were performed by a gas chromatograph (Varian CP, 4900) equipped with a thermal conductivity detector (TCD) and two columns of 10 m, and argon gas was used as a carrier. The gas evolution during the fermentation tests was monitored by using the water-replacement method.

6.5.1.3 Energy Conversion Parameter

In order to score and select the most appropriate pretreatment, the two energy parameters already introduced in Chap. 5 were used. The following expression was applied to evaluate the energy contained in the feedstock (E_{OW}):

$$E_{OW} = LHV_{OW} \cdot TSS_{OW} \tag{6.1}$$

where LHV_{OW} is the Lower Heating Value of the OW (kJ/kg) and TSS_{OW} is the concentration of the substrate evaluated as total suspended solids of the OW in the fermenting broth used (g/L). The initial energy contained in the SPR, SPRex, OWM and CSS was also calculated by applying Eq. 6.1. $LHV_{SPR} = 19{,}591 \pm 50$, $LHV_{SPRex} = 18{,}473 \pm 70$, $LHV_{OWM} = 17{,}721 \pm 65$, and $LHV_{CSS} = 17{,}730 \pm 40$ in kJ/kg were measured by means of a bomb calorimeter.

The second parameter used was *efficacy* (ζ), differently than done in Chap. 5, it takes into account the efficiency of the actual test with pretreatment compared with that obtained without pretreatment. ζ was calculated as follows:

$$\zeta = \eta_P / \eta_{NP} \tag{6.2}$$

where η_P is the efficiency obtained from the pretreated broth and η_{NP} is the efficiency obtained from the broth without any pretreatment. This parameter permits easily evaluation of the effectiveness of the applied pretreatment: if ζ is less than 1, the pretreatment has lowered the efficiency and has likely produced some inhibitory substances, whereas if ζ is approximately 1, the pretreatment has no effect on the bioreaction, and, lastly, if ζ is greater than 1, the pretreatment is able to increase the energy produced as hydrogen compared with the untreated substrate. Therefore, the higher the efficacy value, the greater the effects of the pretreatment on increasing the energy produced in the form of hydrogen, and thus the efficacy is a means of scoring the pretreatment processes.

6.5.1.4 Results and Discussion

Figure 6.2 shows, as an example, the biogas evolution for SPRex, i.e. the OW after extrusion; the biogas starts to produce after 1 day, indicating that a physical pretreatment such as extrusion was useful to remove the packaging and to favor

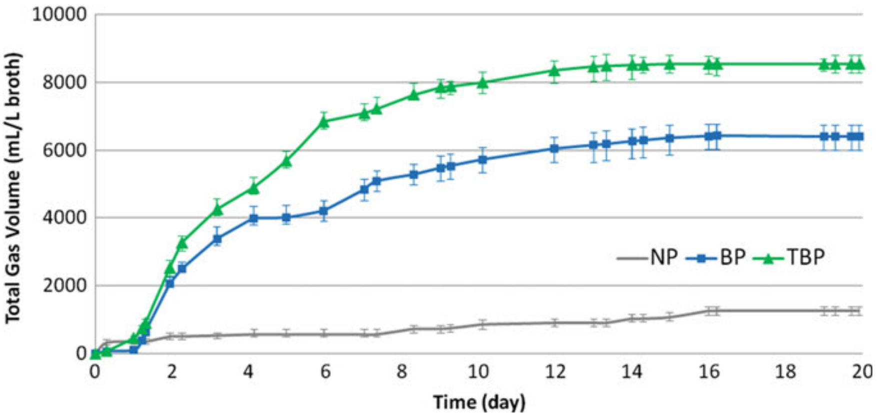

Fig. 6.2 Cumulative gas production ($H_2 + CO_2$) from SPRex with different chemical treatments: none (*NP*), alkaline (*BP*) and thermal alkaline (*TBP*)

hydrolysis. In Table 6.7 the results for all the tests are given (additional results are present in [38]) together with the value of the efficiency and the efficacy.

The shape of the saturation curves with SPRex of biogas production are similar for all the tests. Referring to Table 6.7 of energy produced, in the case of SPR it was noted that AP generates larger amounts of biogas than all the other pretreatments that show values below the NP test. This result is in agreement with the results of [39], which demonstrated that an acid pretreatment significantly increased the bioavailability of cellulose to enzymatic hydrolysis for fresh and processed vegetable refuses from agro-food industry wastes used as feedstock. However, to determine the effectiveness of the pretreatments, it is necessary to evaluate the energy produced through an estimate of the percentage of hydrogen in the biogas and not only the quantity of biogas produced. As a result, BP produces a greater percentage of hydrogen, 32.15 %, which is quite high compared with the values obtained with other pretreatments. In contrast, the results achieved with SPRex show that the pretreatments were able to significantly increase the amount of biogas produced from 1,000 mL of biogas per liter of broth to 8,000 mL/L of broth, with a hydrogen percentage of approximately 40 % v/v (Fig. 6.2), higher than the yields obtained with non-extruded SPR. This result suggests that the extrusion process at 200 atm had a significant impact on the macromolecules, breaking and changing them into smaller molecules that were able to be digested by the microorganisms, and thus greatly increasing the energy efficiency of the system.

The results obtained with OWM refuse show that all the pretreatments induced higher levels of biogas production: from 3.000 to 5,000 mL of biogas was produced per liter of broth. The hydrogen percentages achieved in the biogas are quite different and range between 34 and 51 % for all the situations tested, which means that OWM is suitable for biohydrogen production through anaerobic digestion. If the results are examined in more detail, it is possible to observe that all the pretreatments were able to improve the efficiency and that the highest percentages of hydrogen were obtained when a combined pretreatment (chemical–thermal) was adopted, with TBP_{30min} offering the highest value of 51.6 % v/v of hydrogen in the biogas.

Lastly, as far as CSS is concerned, which is the waste with the highest content of lignocellulosic structures (Table 6.6), two types of pretreatment were used: thermal-basic with different conditions and an ultrasonic pretreatment. It can be seen in Table 6.7 that all the pretreatments considerably increased the production of energy compared with the non-pretreated broth. While higher quantities of gas were obtained for $TBP^{a)}$ and $TBP^{b)}$, UP produced less biogas. Although the ultrasonic pretreatment produced the best results, those obtained with thermal-basic for both the type (a) and type (b) pretreatments, which yielded similar results to those obtained through UP, should not be disregarded. As far as the value of SPR efficacy is concerned, BP shows the best results; the efficacy increased more than six-fold ($\zeta = 6.4$), whereas TBP gave an increase of only 1.4 ($\zeta = 1.4$). In our opinion, the reason for this large decrease in efficacy is driven by the thermal aspect, which constitutes the essential difference between these two pretreatments. The high temperatures caused a type of caramelization of the free sugars, which are present in significant amounts in SPR (more than 17 %; Table 6.6), and this in turn led to a decrease in the available substrate for the microorganisms. Another explanation could be that sugars react to the presence of proteins (6 %; Table 6.6) or amino acids at elevated temperatures, according to the so-called Maillard reaction. This involves a reaction between the aldehyde group of the sugar and the amino group present in the protein, which causes the formation of polymers of higher molecular weight and with dark colors. It is noticeable that in the case of SPR (Table 6.7), AP decreases the efficacy ($\zeta = 0.4$), and this is amplified by temperature ($\zeta = 0$), most likely because the acid is able to liberate some substances that are toxic to the microorganisms. In the case of SPRex, the best efficiency and efficacy were obtained through TBP, with $\eta = 2.4$ % and $\zeta = 17.0$. However, the results with BP were also quite high, $\eta = 1.6$ % and $\zeta = 11.4$; these results were the highest values obtained when all the tested wastes were compared, which means that physical pretreatment performed through extrusion provides excellent results thanks to its capacity to break down the larger molecules and to allow them to dissolve in water, increasing the possibility of being digested by microorganisms. This fact can be corroborated by comparing the results obtained from the broths without pretreatment (NP) of SPR and SPRex, which clearly shows that the efficiency of SPRex is more than 10 times that of SPR: $\eta = 0.019$ and $\eta = 0.14$, respectively. Regarding the OWM, it can be seen in Table 6.7 that the most appropriate pretreatment for greater efficacy was TBP at 30 min, with $\zeta = 9.9$, which shows that the energy produced increases nearly 10-fold with reference to the efficiency obtained with NP. Obviously, in the case of TBP_{30min}, which required more time, more energy was consumed. However, it is also worth noting that the basic pretreatment presents certain other advantages, e.g., during the operation of a plant, as is well known, hydrogen production is accomplished with a concurrent production of liquid metabolites composed of large amounts of VFA, which tend to reduce the pH of the system at the risk of stopping the reaction due to solventogenesis. For this reason, it is necessary to control the pH and to add a base. The basic feed, in the case of continuous operation, therefore leads to savings in the quantity of the base necessary for pH control. Examining Table 6.7, it is possible to state that for refuses with an average content of lignocellulosic material, such as OWM refuse, the best pretreatment is TBP_{30min}. Lastly, for the CSS refuses, once again the results suggest an energy sustainability analysis (Chap. 9) to assess the most energetically sustainable pretreatment between UP, $TBP^{a)}$ and $TBP^{b)}$, which present very similar levels of efficacy. It is important to note that the chemical analysis of biogas in all the tests has shown that the biogas was free of methane, indicating a lack of methanogenic activities. This can be considered an indirect confirmation of the fact that after the acid treatment of anaerobic sewage sludge, the mixed consortium is free of methanogens. As a final consideration, it is important to remark that all the three types of refuse were able to produce hydrogen without the addition of micronutrient or protein supplements to keep the microorganisms alive and that the substrate has a significant influence on the overall hydrogen production.

6.5.2 H_2 Production from Vegetable Wastes in a Laboratory-Scale Bioreactor

The objective of this test with a laboratory-scale bioreactor was to verify the feasibility and reproducibility of the production of H_2. Organic waste market (OWM) was used in the test because of its variation according to the seasons, and its great daily abundance; therefore a different vegetable waste, relative to those used in the previous section, was collected. According to the results obtained previously, alkaline pretreatment was used. A test with glucose was carried out as a comparison.

6.5.2.1 Pretreatments of Substrate

The anaerobic microflora used in this test were the same as that reported in the previous section. The OWM material used was taken from the local fruit and vegetable market. First of all, it was weighed and cut in small pieces, then the materials were finely chopped by a kitchen blade mixer; Fig. 6.3 shows the composition of the biowaste utilized. Initial pH and density were 4.0 and 0.9 g/mL, respectively. The chopped OWM was diluted with tap water (1:1), treated by adding 2 N NaOH to reach pH 12.5 and kept at this level for 24 h at 30 °C.

6.5.2.2 Experimental Test of H_2 Production

The pretreated 1.8 L of broth was inoculated in a ratio of 10 % v/v in a stirred-batch reactor STR (Minifors, Switzerland) as previously described in detail (Chap. 3); anaerobic conditions were obtained by sparging with nitrogen gas at the beginning

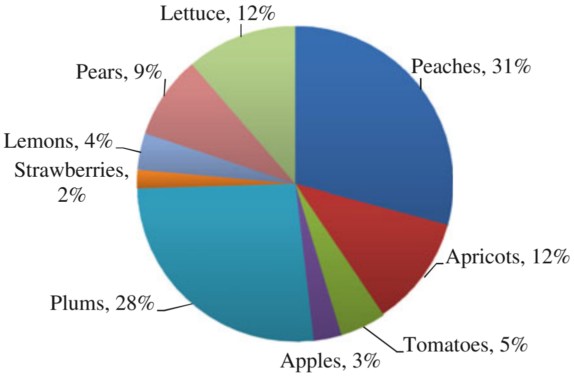

Fig. 6.3 Composition (% w/w) of vegetable and fruit waste used in bioreactor test

of the fermentation. A different stirring power (300 rpm instead of 200) was necessary because of strong, apparently non-Newtonian behavior of the fermenting broth. The initial pH of the media was set to 7 and manually adjusted during fermentation, because of strong viscosity that did not permit good pH measurement. The experiment was conducted at 35 °C.

6.5.2.3 Analyses

Temperature, pH and redox potential were monitored online every minute using Iris NT Software (Infors AG, Switzerland). Liquid samples were taken out of the reactor at different times during the fermentation for the rough determination of sucrose by manual refractometry due to the impossibility of using the enzymatic test for glucose determination. The gas produced during the fermentation was constantly measured by a volumetric gas counter (Milligascounter, Ritter), and then collected in a Tedlar bag (SKC, 231-05 series) and analyzed with a gas chromatograph (Varian, CP 4900).

6.5.2.4 Results and Discussion

As a result of dark anaerobic fermentation over 1 L of vegetable waste diluted in tap water (1:1) produced approximately 10 L of gas containing hydrogen, whose proportion ranged on average from 18 to 42 % (v/v). Table 6.8 gives a comparison of results obtained with OWM and with glucose, both carried out in the same experimental conditions.

The H_2 content in the biogas was lower than that obtained using glucose; these results are in accordance with other studies [31, 34]. The two graphs (a and b) of Fig. 6.4 provide an overview of the parameters monitored during fermentation time. From the first graph (a), one can see on the left *y*-axis the cumulative gas produced (H_2 and CO_2) developed after a few hours, and on the right *y*-axis the flow rate of

Table 6.8 Comparison of bioreactor test with OWM and with glucose

Experimental results	Glucose	OWM
Lag phase (h)	13 ± 2	8 ± 3
Duration of test (days)	14 ± 0.5	6 ± 0.5
Maximum biogas production (L_{H2}/L)	21.3 ± 2	10 ± 0.3
Mean H_2 concentration (% v/v)	47.8 ± 2	30.0 ± 1.5
Maximum H_2 production (L_{H2}/L)	10.2 ± 1.4	3.0 ± 0.2
TS initial (g/L)	58 ± 1.5	31 ± 1.5
TS final (g/L)	0	1.5 ± 0.5
Δ_{TS}	60	31
$Y_R = H_2/\Delta_{RS}$ (mL/g)	170	97
Efficacy *versus* glucose (ζ_{Glu})	100	57

the gas as an experimental derivative of the cumulative gas produced. At first glance, from Fig. 6.4b, one observes a strict relation between biogas evolution, pH and redox developments similar to that found in the glucose test, but the discontinuity of the pH and consequently the redox evolution is also evident. The strong non-Newtonian behavior of the broth impeded automatic control of the pH, and hence it was necessary to control it manually. Due to the consistency of the broth, some problems occurred during the fermentation time course; it caused poor agitation with the formation of lumps of material and these stuck to the pH electrode probe, thus making it impossible to measure the pH and blocking the control loop. On these occasions, manual controls were necessary.

Comparing this test with the one described in Chap. 4, conducted at the same temperature (35 °C) with glucose as carbon source (Table 6.8), one can make the following observations. Despite the presence of more complex substrates, mainly composed of carbohydrates with complex structure and a small percentage of proteins, fats and fibres, the microorganisms show a good ability to utilize vegetable biomass. This is confirmed from a very short lag phase of about 8 h, very similar to that of 13 h in the test with glucose. The short lag phase is certainly facilitated by the alkaline pretreatment that breaks down complex polymers in hydrolyzed monomers. Furthermore, the alkaline pretreatment has other advantages: it might be well adapted to a full-scale plant in which the waste tank of the anaerobic plant could be filled with alkaline solution at the beginning; it does not need direct energy consumption as it occurs with other treatments; and, finally, it also seems very efficacious with different vegetable streams. This probably occurs because the NaOH base is able to destructure the crystallinity of the hemicelluloses and increase the amorphous hemicelluloses, which are more easily attacked by the microorganisms' enzymes. However, because of the heterogeneous and complex nature of celluloses and the contribution of other components, such as lignin and other organic components, it is not the only factor affecting the enzymatic attack. The process is very complicated, because it involves several reactive and non-reactive phenomena, e.g. the dissolution of non-degradated polysaccharides, peeling-off reactions, such as the

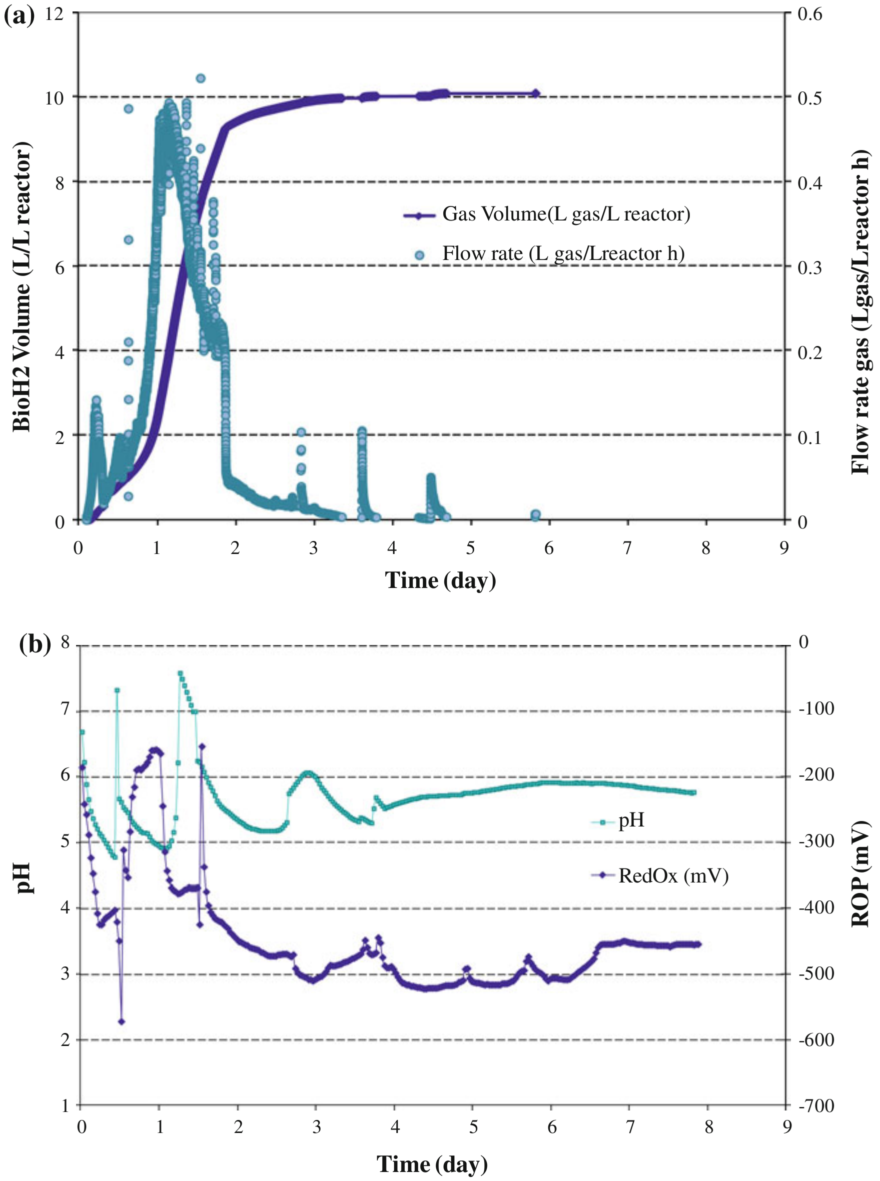

Fig. 6.4 Test of OWM with alkaline chemical treatments at pH 12.5 with 2 N NaOH: **a** cumulative gas production ($H_2 + CO_2$) and flow rate of the gas; **b** pH and redox time course

formation of alkali-stable end groups, the hydrolysis of acetyl groups and glycosidic bonds and the decomposition of dissolved polysaccharides. Another possible explanation for the lower lag phase could be the presence of sugars, together with the large quantity of suspended solids, which creates a support for the microorganisms, increasing the contact surface between the enzymes and the sugars with beneficial effects on the mass transfer phenomena, a problem that is always present in such types of multiphase system, even in the presence of glucose only. As far as H_2 productivity and H_2 production are concerned (Table 6.8), the former is higher than glucose and the latter lower. The available sugars are lower than the glucose equivalent ($\sim$60 g/L), but those present in OWM refuse are more easily converted, shown by the shorter duration of the test. This aspect could also be explained by the mass transfer phenomena previously mentioned.

The rheological behavior of the broth deserves special mention. As experimentally verified (see Fig. 6.4), this behavior is not acceptable in a full-scale bioreactor, and this is one of the most important problems that need to be solved for the scaled-up procedure of such a full-plant application when using organic refuse as substrate to produce energy. As for the energy production, the energy efficacy seems to be a very good parameter to account for this aspect; from Table 6.8, the efficacy is $\zeta = 57\ \%$ of that of glucose evaluated as a ratio of Y_R, but it is more than 11 times the efficiency obtained with Erlenmeyer flasks, indicating that the control of pH and the agitation are able to increase the overall efficiency of the process, even if the problem of mixing remains an open question regarding scale-up. The value of the efficiency of 57 % *vs*. glucose, seems to indicate that there is still a great opportunity to increase the efficacy of the whole process. This result, in the authors' opinion, is a good proof of concept regarding the feasibility of using pretreated vegetable and fruit waste as feedstock for hydrogen production.

6.6 Conclusion

Residual biowastes are suitable substrates for H_2 production. In general, extrusion of SPR provided the best results. The best values of efficiency and efficacy were achieved for TBP, which improved the performance obtained with the broth without pretreatment 17-fold. It is important to note that this type of refuse was extruded at 200 atm to remove packaging. OWM, despite providing one of the best results, is the substrate that produced the highest quantity of energy without any type of pretreatment. This means that among the substrates used, OWM is the most easily digested one; however, the best pretreatment, which improved the energy production 10-fold, was a combination of chemical and thermal processes. As far as SPR is concerned, exposure to high temperatures for a long period appears to be unsuitable for increasing energy production in such substrates, most likely because the caramelization reactions of the sugars hinder digestion by microorganisms. However, it was observed that the BP significantly increased the efficiency with respect to the substrate, with an efficacy value of 6.4. The use of UP, on the other hand, completely destroyed the lignocellulose structures and increased the substrate surface area to a significant extent. The suggestion is that a basic pretreatment, in combination with a thermal one, is able to increase the hydrogen obtainable through the anaerobic fermentation process without the addition of micronutrients or other additives. The choice between a basic pretreatment, a basic/thermal pretreatment, and an ultrasonic one needs to be evaluated through a detailed life cycle assessment of the three options, in which it would be necessary not only to consider the energy consumed directly but also the energy necessary to obtain this energy and the energy necessary to obtain the chemicals. Lastly, a test of OWM with the bioreactor confirms the good opportunity to use OW for hydrogen production even though the problem of mixing the fermenting broth needs to be carefully addressed in the scale-up procedure.

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