



## Hydrogen production: Two stage processes for waste degradation

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### ABSTRACT

The dark fermentation process generates hydrogen by biological means. It presents two main advantages: fulfilling requirements for mild operational conditions and gaining benefit from the residual biomass. The process itself may be seen as a pre-treatment step in a complete stabilisation chain, with the aim of attaining the valorisation of residual biomass. However, increasing the yield of H<sub>2</sub> production is an imperative task. In this manuscript, a review of recent work in the field of fermentative hydrogen production is presented. As dark fermentation has a maximum yield of 33% (on sugars), a description is also presented of possible second stage processes for the degradation of dark fermentation effluents. Alternatives considered were photofermentation and bioelectrochemical systems (BES) as processes capable of converting fermentation sub-products into H<sub>2</sub>. Anaerobic digestion as a final stabilisation stage was also considered owing to the wide application of this technology in the treatment of bio-wastes.

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### 1. Introduction

In recent years, research activities have focused extensively on alternative fuels, with the aim of reducing the high consumption of fossil fuels, either in order to provide local energy resources and thus reduce external dependency or as a means for reducing CO<sub>2</sub> global emissions due to the increasing concern society is experiencing with regard to climate change. The rapid development of “clean fuels” sets an important challenge for two main reasons. First, new fuels are needed to supplement and ultimately replace depleting oil reserves. Second, fuels capable of zero CO<sub>2</sub> emissions are needed to slow the impact of global warming (Rupprecht et al., 2006).

Hydrogen (H<sub>2</sub>) has been suggested as the energy carrier of the future. It is not a primary energy source, but rather serves as a medium through which primary energy sources (such as nuclear and/or solar energy) can be stored, transmitted and utilized to fulfil our energy needs (Das and Veziroglu, 2001). H<sub>2</sub> is considered a clean fuel, since it presents a high energy yield (142.35 kJ g<sup>-1</sup>) and it is water the exclusive product obtained from the combustion of this molecule (Das and Veziroglu, 2001). Although, H<sub>2</sub> has many obvious advantages, the storage and transportation of this gas present several problems still waiting for a solution. Pressurised hydrogen gas takes a great deal of volume compared with other fuels like for example, gasoline that with equal energy content, needs about 30 times bigger volume at 10 MPa gas pressure.

There are also obvious safety concerns with the use of pressurised or liquefied hydrogen in vehicles (David, 2005).

Conventional methods for producing H<sub>2</sub> gas include: steam reforming of methane and hydrocarbons, non-catalytic partial oxidation of fossil fuels and auto-thermal reforming. These methods are all energy intensive processes requiring high temperatures (>850 °C) (Kapdan and Kargi, 2006). In this way, when it is taken into account that a continuous increase is expected in the demand for the use of H<sub>2</sub>, the main goal in the near future should be to attain cost-effective production processes from renewable sources. Among the processes currently available to fulfil this objective, it is worthwhile mentioning the production of H<sub>2</sub> by thermal processing of biomass, as also biomass pyrolysis and gasification through the steam reforming of gases by water–gas shift reactions. These methods present the main advantage of being capable of treating biomass with a high lignocellulosic content, thus avoiding market distortions, which were observed in the past because of an expectation of increasing demand for raw materials.

Biological methods of hydrogen production are preferable over chemical methods because they offer the possibility of using sunlight, CO<sub>2</sub> and organic wastes as substrates. These methods are considered environmentally benign conversions, which take place under moderate conditions (Redwood et al., 2009). The biological methods for generating H<sub>2</sub> include light-dependent methods, such as direct and indirect biophotolysis and photo-fermentation. The other routes for biological production are not light-dependent methods. In this category are included the dark fermentation process, bioelectrochemical systems (BES) and water–gas shift reaction mediated by photoheterotrophic bacteria. However, the need of light of this latter process for microbial growth and the use of

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**Table 1**  
Advantages of the dark fermentation process.

Advantages of the dark fermentation process	References
High production rates in terms of volume of the reactor, independence of light making possible the production through the whole day	Das and Veziroglu (2001)
Many different substrates can be fermented to produce H <sub>2</sub>	Kapdan and Kargi (2006)
The anaerobic degradation of organic matter by heterotrophic microorganisms can liberate H <sub>2</sub> at high rates	Redwood et al. (2009)
The use of mixed cultures allows stable operation when wastes are used as substrate under continuous operation	Bartacek et al. (2007)
There is no oxygen limitation problem since it is an anaerobic process	Nath and Das (2004)
Inoculation of the anaerobic reactors for hydrogen production has been successfully attained without any pre-treatment and without inoculum addition	Kim et al. (2009), Lin and Jo (2003) and Gómez et al. (2009)

CO as a carbon source may seem to be technical and economic barriers with regard to the reactor design and the thermal pre-treatment step necessary for the generation of CO. Ustak et al. (2007) compared two different biological methods for hydrogen production: fermentative and photosynthetic based upon the modality of batch cultures. For testing fermentative bio-hydrogen production, four mixed cultures representing anaerobic microorganisms (dominant strain *Clostridium*) were selected. The testing of green algae proved that the most effective was the algae species *Scenedesmus*. High bio-hydrogen purity was analytically verified. The fermentative method of H<sub>2</sub> production was more efficient; it does not need light, has a longer efficiency of a single charge and enables effective use of different biological wastes.

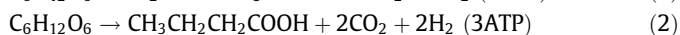
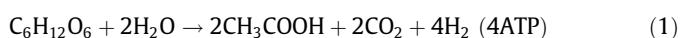
However, production of hydrogen from either of the methods previously commented upon is characterised by low efficiency, this acting as a barrier for commercial application. Several disadvantages of these methods have been stated by Das and Veziroglu (2001). A fact of great relevance is the small scale at which most of these technologies have been tested, with the dark fermentation process being the only one with a prospect of being rapidly scaled-up owing to its similarity to the well-known anaerobic digestion process. In addition, this process can use residual carbohydrate-rich biomass as substrate, which makes the process an alternative for the conventional biological methods for treating and taking full advantage of this biomass. The process itself may be seen as a pre-treatment step in a complete stabilisation chain.

In the present review, a description of successful experiences for the production of hydrogen through dark fermentation from residual biomass was undertaken. As dark fermentation has a maximum yield of 33% (on sugars), a description is also presented of possible second stage processes for further degradation of dark fermentation effluents (mainly containing fatty acids). Alternatives for the second stage considered were photofermentation and BES as processes capable of converting fermentation sub-products into H<sub>2</sub>. Anaerobic digestion as a final stabilisation stage was also considered, owing to the wide application of this technology in the treatment of bio-wastes.

## 2. Fermentative hydrogen production

The evolution of hydrogen by fermentation has several advantages over other biological methods. Some of these advantages are listed in Table 1. In addition, it should also be pointed out the easiness of scaling-up because of its similarities to the well-known anaerobic digestion process and thanks to the experience gained in the industrial application of this technology.

Dark-fermentation processes produce a mixed biogas containing primarily H<sub>2</sub> and carbon dioxide (CO<sub>2</sub>). The route for producing H<sub>2</sub> when acetate and butyrate are the main sub-products was described by Thauer et al. (1977).



**Table 2**  
Most common pre-treatments applied for obtaining H<sub>2</sub> producing microflora.

Pre-treatment	References
Inoculum heat shock	Khanal et al. (2006), Perera and Nirmalakhandan (2010)
Acidification, basification	Wang et al. (2006), Chang et al. (2011)
Loading shock	O-Thong et al. (2009), Luo et al. (2010)

The process is then characterised by low efficiency and this also becomes the main limiting factor for commercial application. Only 33% of the chemical oxygen demand (COD) contained in the waste can be transformed into hydrogen (considering glucose). The remainder is mainly composed of volatile fatty acids (VFAs – acetate, butyrate) (Bartacek et al., 2007). Nonetheless, the formation of caproate has also been reported in dark fermentation systems, with the presence of this acid being coupled to the production of H<sub>2</sub>. The generation of caproate does not translate into a higher H<sub>2</sub> yield since its formation has been explained by the secondary fermentation of two substrates, either ethanol and acetate or ethanol and butyrate. Thus, the appearance of caproate in the final products is an indication that significant solventogenesis has occurred and thus the yield of the fermentative H<sub>2</sub> production is poor (Ding et al., 2010). Thus, considering the composition of the effluent stream of the dark fermentation process, direct biological production of H<sub>2</sub> appears to be restricted to a pre-treatment step in a larger bio-energy or biochemical production concept (Angenent et al., 2004).

### 2.1. Operating conditions for improving H<sub>2</sub> yields

Strategies for improving H<sub>2</sub> yields have been reviewed by Kramer and Bagley (2007) analysing diverse alternatives, such as inoculum pre-treatment, gas sparging, reduction of H<sub>2</sub> and CO<sub>2</sub> levels in the liquid phase, and varying organic loading rates. Several options successfully tested for obtaining active hydrogen producing microflora are listed in Table 2. These pre-treatments may regularly be applied to reactors in order to maintain the activity of H<sub>2</sub> producing microorganisms. However, when considering large scale implementations, some of these pre-treatment options may increase operating costs, as it is the case of heat shock on starting-up the reactor or from ongoing heat treatment as proposed by Khanal et al. (2006) for maintaining an active H<sub>2</sub> producing population during extended operation. In addition, differences in hydrogen yields have proved to disappear in continuous experimentations which indicate that pre-treatment methods have only short-term effects on hydrogen production (Luo et al., 2010).

Another relevant aspect that should be considered is the inhibition caused by the accumulation of fermentation products, with this being detrimental to H<sub>2</sub> yield. Operating alternatives associated with the removal of such products will favour process efficiency. In this sense, either gas sparging or vigorous mixing will

**Table 3**

Dark fermentation process: reactors operating under continuous conditions.

Substrate	Reactor	HRT	H <sub>2</sub> yield	OLR	References
Molasses	ABR(27.5 L) Mesophilic	13.5 h	0.13 L/gMLVSS d	8.89 kgCOD/m <sup>3</sup> d	Li et al. (2007)
Vegetable kitchen waste	CSTR (20 L), Thermophilic	4 d	0.9–1.7 mmol H <sub>2</sub> /g COD	19–28 g COD/L*d	Lee et al. (2010b)
Waste sugar	CSTR (30 L), Mesophilic	20–12.5 h	0.94–1.78 mmol H <sub>2</sub> /mol on COD input	2–14 kg VS/m <sup>3</sup> d	Krupp and Widmann (2009)
Molasses	CSTR (1480 L), Mesophilic	10.57–3.9 h	26.13 mol H <sub>2</sub> /kg COD <sub>removed</sub>	3.11–85.57 kg COD/m <sup>3</sup> reactor/d	Ren et al. (2006)
Sucrose	CSTR (400 L), Mesophilic	12 h	1.01 mol H <sub>2</sub> /mol sucrose	1.6 g COD/L*h	Lin et al. (2010)
Sucrose	Agitated granular sludge bed bioreactor (400 L)	12–4 h	0.97–1.04 mol H <sub>2</sub> /mol sucrose	40–240 kg COD/m <sup>3</sup> /d	Lin et al. (2010b)

aid in reducing H<sub>2</sub> and CO<sub>2</sub> concentrations inside the reactor (Kraemer and Bagley, 2006) and thus favour stability for extended operating periods. Gómez et al. (2009) reported stable operation during continuous fermentation of household waste when mixing was provided to the reactor in contraposition to what occurs in static reactors. This same effect may be attained by gas sparging inside the reactor. Nguyen and co-workers (Nguyen et al., 2010) demonstrated that the removal of the H<sub>2</sub> produced from the gas headspace during batch fermentation improved H<sub>2</sub> yields. However, accumulation of VFAs still poses a problem. An increase in the organic loading rate, in an attempt to increase the treatment capacity of reactors may lead to inhibition of the fermentation system, owing to VFA accumulation. Wang and Zhao (2009) demonstrated that a significant reduction in H<sub>2</sub> yield was caused by an increase of the OLR to 30.2 kg VS m<sup>-3</sup> d<sup>-1</sup>, when food wastes were treated as the substrate. In this way, adaptation of the inoculum may play a crucial role in attaining stable operation at high concentrations of VFAs, as demonstrated by Valdez-Vazquez et al. (2005). These authors reported successful performance from a reactor treating organic wastes during solid state fermentation.

The commercialization of industrial hydrogen fermentation makes imperative to achieve steady operation and also to carry out the process under non-sterile conditions using readily available complex feed stocks with only minimal pre-treatment. Microbial consortia may address these issues if they are selected for growth and dominance under non-sterile conditions (Hallenbeck and Ghosh, 2009). Steady operation over long periods of reactors producing H<sub>2</sub> has been reported by several authors (Valdez-Vazquez et al., 2005; Chu et al., 2008). However, fluctuating H<sub>2</sub> production has also been reported. This is the case of results obtained by Zhu et al. (2008) when fermenting potato waste in a two-phase configuration. Similar results were reported by Gómez et al. (2009) when fermenting food waste under static conditions. These divergences in results obtained may indicate that the selection of optimum parameters is a key factor for attaining stable performance.

## 2.2. Laboratory and pilot scale fermentation experiences

Centralised collection of urban solid wastes and chemical characteristics of the organic fraction contained in these wastes are two factors that make them a suitable substrate for the dark fermentation process. In addition, the fermentation of wastes for H<sub>2</sub> production may be considered as a pre-treatment option which may be integrated with minimum modifications into centralised solid waste treatment plants where an anaerobic digester is already operating. On these lines, fermentation of household wastes has been studied by several authors (Gómez et al., 2009; Liu et al., 2008; Kim et al., 2009; Lee et al., 2010) under different temperature conditions, increases in H<sub>2</sub> yields being reported when there were increases in temperature to thermophilic regimes.

Substrates such as wastewaters with high carbohydrate content have also been demonstrated to be suitable for the dark fermenta-

tion process. This is the case for molasses (Li et al., 2007) and cheese whey (Ferchichi et al., 2005), which have been studied using completely stirred tank reactors (CSTRs) and reactors with immobilised biomass. Another feedstock of interest is the use of marine algae. Jung et al. (2011) tested various algae for fermentative hydrogen production, with *Laminaria japonica* presenting the highest H<sub>2</sub> yields. The presence of particulate material set limits to the application of high-rate systems. For this reason CSTR systems are often applied for the treatment of household wastes, while in general, attached growth systems are used for the fermentation of high strength wastewater. Immobilized systems are an effective and stable approach for continuous hydrogen production allowing efficient utilization of carbon substrates (Jo et al., 2008).

Experiments with dark fermentation processes are rapidly increasing on a laboratory scale. Several reports in litter-scale can be found in literature. Table 3 shows a list of published results for different fermentation systems working under continuous operation in reactors of size bigger than 20 L. Although results are promising, on feature that needs to be addressed is the requirement for alkalis. Controlling the pH of the process requires large amounts of alkaline solutions, which not only increases operating costs, but also leads to an effluent with high conductivity. When concentrated solutions of NaOH are used, the effects on a potential second treatment process should be considered.

Kraemer and Bagley (2005) proposed the recycling of a fraction of the methanogenic effluent in order to take advantage of the alkalinity generated in the digestion phase which is needed in the dark fermentation phase for pH control. Although results reported were not satisfactory, since lower H<sub>2</sub> productivity was attained during recycling, this strategy has been tested with satisfactory results by other authors. This was the case for the two-phase process (thermophilic-mesophilic) with sludge recirculation tested by Lee et al. (2010) in a 10 L H<sub>2</sub> reactor and a 40 L CH<sub>4</sub> reactor. Addition of precipitated sludge at the bottom of a storage tank was used for pH control. These authors obtained stable operation over a period of 150 days with a H<sub>2</sub> yield of 205 mL g<sup>-1</sup> VS added. This configuration presented two main advantages. The first was that the hydrogen-producing bacteria which exist in digested sludge could replenish the hydrogen production reactor by recirculation. The second advantage was that acidity in the hydrogen production stage was neutralized by recirculation of the digested sludge, which presented high alkalinity, so that the reagent for pH adjustment could be saved.

Another option for reducing the amount of alkaline solutions needed would be the use of co-substrates. The co-fermentation of manures and carbohydrate-rich wastes would avoid acidification of the reactor, although high VFA concentrations might occur. However, single fermentation of swine manure resulted in low to negative H<sub>2</sub> yields. There are several factors that may contribute to low hydrogen production from swine manure, such as the lack of suitable sugars in the wastewater, since most of the hydrogen evolved in high-rate hydrogen fermentation tests is a result of sugar in the sample. It appears that hydrogen recovery from swine

wastewater will not be feasible by fermentation processes unless some breakthrough is made in changing the nature of the wastewater or the conditions for microbial growth that inhibit the utilization of  $H_2$  by microorganisms in the wastewater (Wagner et al., 2009). On these same lines, Perera and Nirmalakhandan (2010) obtained successful results under batch conditions when co-fermenting sucrose with heat-treated cattle manure. These authors reported a 10% improvement in the  $H_2$  yield and also demonstrated the capacity of these wastes to reduce buffering needs. Zhu et al. (2009) studied the fermentation of swine manure supplemented with glucose in a 4 L working volume reactor under mesophilic conditions at varying pH and HRT. These authors reported that to increase hydrogen content in the offgas, methane production had to be limited below 2%. On the basis of the results recorded above, it seems reasonable to assume that different strategies intended to increase fermentation yields and lower operating costs should also consider the use of acid-rich effluents, either to enhance treatment efficiency or to make the overall process economically viable by the generation of a high-value product in a second fermentation stage (Mohan et al., 2010).

### 3. Photofermentation

Increasing  $H_2$  yields from dark fermentation processes is a challenge. Experience shows that single-stage processes are not efficient from the point of view of hydrogen yield, since only part of feedstock is converted to hydrogen (Modarresi et al., 2010). Sub-products obtained in this process may be further metabolised to produce additional  $H_2$ . This can be achieved by means of photofermentation. Purple non-sulfur (PNS) photosynthetic bacteria constitute a non-taxonomic group of versatile organisms which can grow as photoheterotrophs, photoautotrophs or chemoheterotrophs (Basak and Das, 2007). PNS bacteria produce  $H_2$  under photoheterotrophic conditions (light, anaerobiosis, organic electron donor) (Redwood et al., 2009). The advantages of this process, relative to biophotolysis of water using green algae and cyanobacteria, are that oxygen does not inhibit the process and that these bacteria can be used in a wide variety of conditions (i.e. batch processes, continuous cultures, and immobilized systems) (Holladay et al., 2009).

The overall biochemical pathway for the production of  $H_2$  by the photofermentation process was described by Das and Veziroglu (2001). Nitrogenase is the enzyme responsible for producing  $H_2$  from protons and simultaneously fixing nitrogen in the PNS bacteria.  $H_2$  production by nitrogenase is an irreversible reaction and four ATPs are required per mole of  $H_2$  produced, making this reaction energy inefficient (Basak and Das, 2007). The culture medium should be under a nitrogen limitation (i.e. a high C/N ratio), which forces the bacteria to 'dump' the excess energy and reduce power through the production of  $H_2$  (Koku et al., 2002). In this sense, coupling systems for dark and light fermentations may seem possible only for treating substrates characterised by a low C/N ratio. This state of affairs means that it is unsuited for the treatment of mixed substrates intended to increase  $NH_4^+ - N$  concentrations in the reactor as a means for controlling pH in the dark fermentation system, as also the previously mentioned option of adding high N content co-substrates. This should be added to the obvious problem of keeping the  $H_2$  producing microflora separate.  $NH_4^+ - N$  may be naturally presented in wastewater and may also be generated in the dark fermentation processes when the HRT is high enough to achieve protein degradation. This would be especially so in the case of the fermentation of particulate substrates, such as food wastes, which may need an HRT of as long as 5 days. The presence of  $NH_4^+ - N$  inhibits  $H_2$  synthesis by repressing the expression and activity of nitrogenase, the enzyme catalysing  $H_2$  production in

PNS bacteria (Li et al., 2010). Although some constraints need to be overcome, a sequential process with dark fermentation and photofermentation stages allows an increase in  $H_2$  yields and the production of a high quality stream from a bioprocess (Claassen et al., 2010).

Combined dark and photofermentation was studied by Nath et al. (2005) using glucose as substrate. The spent medium from the dark process (containing unconverted metabolites, mainly acetic acid) underwent photo-fermentation by *Rhodobacter sphaeroides* strain O.U.001 in a column photo-bioreactor. Results obtained demonstrated that this combination could achieve higher yields of hydrogen by complete utilization of the chemical energy stored in the substrate. Liu et al. (2010) studied a sequential process using glucose as substrate and an immobilized system for the photofermentation step evaluating key factors, such as: diluted ratio of dark fermentation effluent, ratio of dark and photofermentation bacteria, light intensity, and light:dark cycle. During this combined process, the maximum total hydrogen yield was  $5.374 \text{ mol-}H_2 \text{ mol-glucose}^{-1}$ . However, the sterilization step applied in this case to the dark fermentation effluent may pose a constraint to any scaling-up of the process.

Another important aspect is the effect on photo-fermentation of acetate (HAc) and butyrate (HBU) which are the major soluble products from acidogenic dark fermentation. Chen et al. (2008) studied the photofermentation process using an indigenous PNS bacterium (*Rhodospseudomonas palustris* WP3-5) to produce hydrogen phototrophically. From their results, it was concluded that  $H_2$  yields could be adversely affected by high HBU concentrations (above 2500 mgCOD/L). Moreover, their results also suggested that HBU concentration displayed a more significant impact on  $H_2$  yield when compared to HAc. Although this constraint may affect the overall efficiency of a sequential fermentation process, several studies have demonstrated that a clear improvement in  $H_2$  production can be attained. This was the case for the results reported by Zhu et al. (2010) using enzymatically pre-treated corn stover. Similar results were also obtained by Yang et al. (2010) using acid-pre-treated corncob. These authors reported 90% COD removal from the two-stage process with a consumption of  $98.6 \pm 0.1\%$  of HAc,  $99.3 \pm 0.1\%$  of HBU and 100% of propionate.

One main disadvantage of the light dependent processes is the more complex design of reactors, owing to the need to maintain a suitable proportion between reactor surface area and volume when scaling-up. When the reactor size is increased, light availability may be poor in deep regions of the reactor. In addition, when experiments are carried out under natural sunlight, this will translate into cycles of light and darkness. These two aspects may favour a switch to a fermentative type of metabolism, causing the consumption of substrate with no  $H_2$  evolution either during dark periods or in dark zones of the reactor (Koku et al., 2002). Another aspect related to this consideration is the self-shading effect of cultures which seriously limits the abundance and homogeneous distribution of light. The effect of light/dark cycles on the production of  $H_2$  was investigated by Li et al. (2011) using *R. sphaeroides* ZX-5. These authors reported little or no hydrogen production during dark periods with an immediate recovery in hydrogen production once illumination was resumed.

### 4. Microbial electrolysis cells for hydrogen production

Bioelectrochemical systems (BESs) are emerging technologies which use microorganisms to catalyse the reactions at the anode and/or cathode (Hamelers et al., 2010). Microbial electrolysis cells (MEC) were first developed by Liu et al. (2005) for  $H_2$  production. MECs allow the transformation of organic matter through the oxidation of molecules mediated by microorganisms with the aid of

an external circuit containing a power supply. Oxidation of organic material at the anode results in protons, CO<sub>2</sub> and electrons which are transported through the external circuit to the cathode. These electrons combine at the cathode with protons evolved from the oxidation of the organic matter producing H<sub>2</sub> gas (Jeremiassé et al., 2010). The main advantage of the process is related to its low energy consumption since an applied voltage as low as 0.2 V is considered to be necessary for microbial electrohydrogenesis to produce hydrogen (Hu et al., 2009) in comparison to the theoretical minimum voltage of 1.23 V required for water electrolysis (Rozendal et al., 2007). Extensive research has been performed related to the application of this technology for the production of H<sub>2</sub> by the use of synthetic media (Rozendal et al., 2007; Selembo et al., 2009a; Tartakovsky et al., 2009) and recently using wastewaters (Wagner et al., 2009).

The combination of the dark fermentation process and MEC was tested by Lu et al. (2009) using molasses as substrate. The fermentation was carried out in a CSTR and the acid effluent generated was used as feed stream for the MEC. The combination of the systems resulted in a production rate of 2.11 m<sup>3</sup> H<sub>2</sub> m<sup>-3</sup> d<sup>-1</sup>. Although the presence of methanogens was detected during the performance of the system, repression of methanogen growth could be achieved by the exposure of the cathode to air after each short cycle. Since methanogens can form a biofilm over any part of the cell surface, preventing the growth of these microorganisms is challenging. In addition, a problem that also needs to be solved is the necessity to increase the pH of the fermentation effluent to be treated in the MEC system. H<sub>2</sub>-producing butyrate–acetate metabolism points to a pH range 5.2–5.8 as the optimum over a variety of HRT (6–32 h) and substrate types (sucrose, starch and beer industry wastes) (Hawkes et al., 2007), while the pH for MEC systems should be close to neutrality. This leads to increases in the volume of alkaline solution needed for controlling the pH of two-stage systems, which in turn increases the concentration of salts in the treated effluent. This fact is of major relevance since, when dealing with waste streams, the chemical and biological quality of the treated effluent in a crucial parameter.

Lalauette et al. (2009) also studied a two-stage system (dark fermentation – MEC) with acid-pretreated corn stover as substrate. Higher H<sub>2</sub> yields were reported for the two-stage system in comparison to dark fermentation as a single stage. However, CH<sub>4</sub> was reported to be a problem in all MEC tests performed. These authors stated that the duration of the cycle was the main factor, affecting the cell performance. Their results were coincident with those reported by Lu et al. (2009) also indicating that long cycle times favoured the growth of methanogens.

One aspect that may seem a disadvantage in MEC systems is the use of membranes to separate the cathode and anode compartment, thus developing a pH gradient (Rozendal et al., 2006). The creation of a pH gradient increases the theoretical voltage needed to drive the MEC. While elimination of this membrane may circumvent the problem, it may also cause a substrate/product cross-over giving unwanted side reactions and products (Hamelers et al., 2010). Among the undesired reactions is the production of methane, which occurs particularly when single-chamber systems are used, not keeping the gases, generated at the cathode, separated from the anode (Wang et al., 2009). Methane production in sin-

gle-chamber MECs is primarily associated with current generation and hydrogen gas production, and not acetoclastic methanogenesis. Methane generation will therefore be difficult to control in mixed culture MECs that produce high concentrations of hydrogen gas. By keeping cycle times short, as demonstrated by Lu et al. (2009), and using higher applied voltages ( $\geq 0.6$  V), it is possible to reduce methane gas concentrations (<4%) but not to eliminate methanogenesis in MECs (Wang et al., 2009).

Single-chamber MEC configurations have been studied by several authors (Call and Logan, 2008; Hu et al., 2009; Lu et al., 2009; Tartakovsky et al., 2009). High cathodic hydrogen recoveries have been obtained in single-chamber configurations, with production rates being higher than those obtained in two-chamber MECs. The absence of a membrane allows simplification of the reactor design as well as a reduction in the cost. However, as disadvantage, methane production may inevitably be produced and thus negatively affect H<sub>2</sub> production rates (Wagner et al., 2009).

Before bench-scale reactors can be upgraded to economically feasible applications, the previously mentioned hurdles that limit the overall MEC performance need to be overcome, and efficient and cost-effective cathodes developed, thus allowing a reduction in the cost of reactor materials (Clauwaert et al., 2008). Extensive research has recently been carried out to develop new cathodes, such as those using stainless steel and Ni-alloy (Selembo et al., 2009b), electrodeposited NiW and NiMo (Hu et al., 2009), or Ni electrodeposition onto porous carbon paper (Hrapovic et al., 2010). Although great efforts are being made to develop this technology, and successful and promising results are also being obtained, laboratory scale equipment is still in its infancy, with reported experiences being at a sub-liter scale (Clauwaert et al., 2008).

## 5. Anaerobic digestion process

When the treatment of wastes is being considered, the dark fermentation process would need a final stabilisation stage. The aim of this final stage is to reduce the potential of organic matter to putrefy. This is of great relevance when particulate organic materials are being used as substrate. The two-phase fermentation process for harvesting H<sub>2</sub> in the first phase and methane in the subsequent step may become an economically feasible alternative in the near future for treating residual biomass and also attaining the aim of reducing pollution. The first published report describing this two stage process was presented by Wang et al. (2003). Several authors have continued studying this process (Zhu et al., 2008; Wang and Zhao, 2009; Giordano et al., 2011) and recently, substrates with high content of lignocellulosic material have also been tested in a two-stage configuration, with an acid hydrolysis pre-treatment being necessary in this case (Lakaniemi et al., 2011). These results establish a new line of opportunities for the utilization of a wide variety of substrates.

Table 4 shows some successful experiments reported by different authors. Results of particular importance are those reported by Wang and Zhao (2009) using a pilot scale unit for testing a hydrolysis/acetogenesis rotating drum for H<sub>2</sub> production followed of a methane fermentation reactor (200 and 800 L of working volume respectively). The process was studied under mesophilic condi-

**Table 4**  
Some experiences treating food wastes as substrate and operating at two phase configuration: dark fermentation + anaerobic digestion.

Reactor	HRT (H <sub>2</sub> phase)	H <sub>2</sub> yield	OLR (H <sub>2</sub> phase)	References
Rotating drum (200 L) + CSTR (800 L), mesophilic	240–96 h	0.049–0.065 m <sup>3</sup> H <sub>2</sub> kg <sup>-1</sup> VS	22.7–37.8 kg VS m <sup>-3</sup> d <sup>-1</sup>	Wang and Zhao (2009)
CSTR (10 L) + CH <sub>4</sub> reactor with suspended media (40 L), thermophilic–mesophilic	1.3 d	0.205 m <sup>3</sup> H <sub>2</sub> kg <sup>-1</sup> VS	38.4 kg VS m <sup>-3</sup> d <sup>-1</sup>	Chu et al. (2008)
CSTR (10 L) + CH <sub>4</sub> packed reactor (40 L), thermophilic	3.8–1.28 d	0.056–0.118 m <sup>3</sup> H <sub>2</sub> kg <sup>-1</sup> VS	12.4–37.0 kg VS m <sup>-3</sup> d <sup>-1</sup>	Lee et al. (2010c)
CSTR (500 L) + UASB (2300 L), mesophilic	66–21 h	0.62–3.9 L H <sub>2</sub> m <sup>-3</sup> d <sup>-1</sup>	7.4–71.3 g COD L <sup>-1</sup> h <sup>-1</sup>	Lee and Chung (2010)

tions using as inoculum indigenous mixed microbial cultures contained in food waste, thus avoiding the application of pre-treatments, which over time allows operating costs to be reduced. The maximum gas yield reported by these authors was  $0.065 \text{ m}^3 \text{ H}_2 \text{ kg}^{-1} \text{ VS}$  and  $0.546 \text{ m}^3 \text{ CH}_4 \text{ kg}^{-1} \text{ VS}$  for the  $\text{H}_2$  and  $\text{CH}_4$  producing phase respectively.

Thermophilic operation has been reported to increase  $\text{H}_2$  yields, in comparison to mesophilic counterparts. Extreme thermophilic fermentations tested have been reported to present a predominance of the acetate pathway, which can give higher  $\text{H}_2$  yields than butyrate type fermentation (Kongjan and Angelidaki, 2010). On this line, Chu et al. (2008) working with a two-stage configuration (thermophilic  $\text{H}_2$  reactor – mesophilic  $\text{CH}_4$  reactor) reported gas yields of  $0.205 \text{ m}^3 \text{ H}_2 \text{ kg}^{-1} \text{ VS}$  and  $0.464 \text{ m}^3 \text{ CH}_4 \text{ kg}^{-1} \text{ VS}$ .

## 6. Conclusions

Dark fermentation presents similarities with its homologous digestion process. The experience gained with this process may provide a technical basis for the development of large-scale prototypes dedicated to the production of  $\text{H}_2$ . At present, digestion may be considered as a second stage of the dark fermentation process with the capacity of degrading particulate material. However, increasing  $\text{H}_2$  production yields is an imperative task. Photofermentation and bioelectrochemical systems may be considered as alternatives capable of attaining this goal. However, in the current state, the design of reactors is complicated and no cost-effective approaches have been developed yet.

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