

# FERMENTATIVE HYDROGEN PRODUCTION - PROCESS DESIGN AND BIOREACTORS

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Substitution of fossil fuels with alternative energy carriers has become necessary due to climate change and fossil fuel shortages. Fermentation as a way of producing biohydrogen, an attractive and environmentally friendly future energy carrier, has captured increasing attention in recent years because of its high H<sub>2</sub> production rate and a variety of readily available waste substrates used in the process. This paper discusses the state-of-the-art of fermentative biohydrogen production, factors affecting this process, as well as various bioreactor configurations and performance parameters, including H<sub>2</sub> yield and H<sub>2</sub> production rate.

**Keywords:** biohydrogen production, fermentation, bioreactors, operational parameters, hydrogen yields

## 1. INTRODUCTION

Energy is a factor significantly influencing the development of civilization. In 2010, its world consumption rose by 5.6 % compared to previous year and equaled 12,002.4 mln tonnes oil equivalent. 87 % of the used energy was generated from fossil fuels (33.5 % crude oil, 29.6 % coal, 23.8 % natural gas) (Statistical Review of World Energy, 2011). If the global population increases by 1.4 billion people over the next 20 years, and assuming an economic growth rate of 3.7%, it has been assessed that the global energy demand will rise by 39 % by 2030 (Statistical Review of World Energy, 2011), whereas in 2100, it can reach a value 3.5 times higher than nowadays (Kruse et al., 2005). Analysing the data concerning fossil fuels, it seems that their reserves are not sufficient to meet the energy demand by the end of 21st century (Ball and Wietschel, 2009). Therefore, it is necessary to research and develop energy production technologies based on alternative sources. An important cause for these actions is also the unequal distribution of fossil fuels. The demand for this type of fuels in developed countries forces them to import these fuels without having any influence over their price. The intention of developed countries to become independent from the uncertain import of energetic materials, along with the fact that fossil fuels are the main source of pollution and global climate changes, has become an additional incentive to search for new, renewable energy sources.

In the future, hydrogen can become an important energy carrier as it can be obtained from water electrolysis, pyrolysis, and biomass gasification, methanol and ethanol reforming, as well as biological decomposition of water and organic compounds (Balat, 2008; Demirbas, 2011; Ni et al., 2007; Palo et al., 2007; Stodolny and Łaniecki, 2009; Waligórska and Łaniecki, 2005). Taking into account the current state of the art, hydrogen production by fermentation, using wastewater, cellulose biomass and solid waste from various industry branches is a promising biological method.

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## 2. METABOLISM OF FERMENTATIVE HYDROGEN PRODUCTION

Fermentation is an anaerobic process, in which hydrogen can be obtained from carbohydrate-rich substrates. This process, easier and cheaper compared to other biological methods, allows to obtain a high rate of hydrogen production. However, its bottleneck is low hydrogen production yield due to the creation of reduced organic compounds (Lee et al., 2008 b).

During glycolysis, carbohydrates are decomposed to pyruvate, and its further fermentation depends on the type of bacteria (Hallenbeck, 2009). In the presence of facultative anaerobes (*Enterobacter*, *E. Coli*, *Klebsiella*), pyruvate is converted by means of pyruvate: formate lyase to acetyl-CoA and formate. An increase in the formate concentration leads to a *pH* drop and induction of the formate: hydrogen lyase, which catalyses its decomposition into CO<sub>2</sub> and H<sub>2</sub>. One glucose molecule can be converted into two formate molecules, so the maximum hydrogen production yield is 2 moles per mole glucose (Hallenbeck, 2009). Strict anaerobes (*Clostridium*, *Ethanoligenens*) convert pyruvate into acetyl-CoA and CO<sub>2</sub>, also producing reduced ferredoxin (Fd<sub>red</sub>), which transfers electrons to [FeFe] hydrogenase catalysing the generation of hydrogen. It produces 2 moles H<sub>2</sub> per mole of glucose. Additional quantities of hydrogen can also be produced thanks to the activity of NADH: ferredoxin oxidoreductase, resulting in the oxidation of NADH and production of reduced ferredoxin, which can be subsequently used for proton reduction (Hallenbeck, 2009). Assuming that glycolysis results in the production of two moles of NADH, one could potentially gain two extra moles of hydrogen from this pathway. Therefore, *Clostridium* bacteria are capable of producing 4 moles of hydrogen per mole of glucose (Angenent et al., 2004). The redox potential of hydrogen (-0.42 V, at *pH* 7) is, however, much lower than that of NADH/NAD pair potential (-0.32 V), hence the production of hydrogen using electrons from NADH is possible only at a very low partial pressure of hydrogen (less than 10<sup>-3</sup> atm.) (Angenent et al., 2004; Hallenbeck, 2009).

It is worth noting that from an evolutionary point of view, fermentation is a process which enables organisms to gather energy (ATP) through substrate-level phosphorylation, a thermodynamically controlled process. It has been concluded that regardless of the environmental *pH* the three reactions in which ATP is created have large negative values of free energy: glycolysis, acetate production and butyrate production (Lee et al., 2008 b). In fermentation, reducing power (in the form of NADH) is created as well. Oxygenation of NADH also takes place during the production of lactate, propionate, ethanol and butyrate. Therefore, fermentative bacteria often conduct a mixed acid fermentation, during which acetate, butyrate, lactate, ethanol, formate, succinate, as well as acetone, butanol or butanediol can be produced (Hallenbeck, 2009; Lee et al., 2008b; Muraraka et al., 2008). The ratio of products depends on the type of used microorganisms, the substrate and the conditions of the process (Cai et al., 2011). In practice, hydrogen production efficiency is almost two times lower than its theoretical value (Hallenbeck, 2009; Lee et al., 2011; Wang and Wan, 2009). The goal of future research has to be an increase of this efficiency through minimising the amount of organic products and streaming electrons into hydrogen production pathway (Lee et al., 2010).

## 3. OPERATIONAL PARAMETERS

The operational parameters such as inoculum type, *pH*, temperature, feedstock, H<sub>2</sub> partial pressure are crucial to the performance of fermentative hydrogen production and are reviewed in this section.

### 3.1. Microorganisms

The conditions of dark fermentation have been the subject of long-term investigations. Hydrogen can be produced by strict anaerobes from the *Clostridiaceae* family (e.g. *C. butyricum*, *C. actobutylicum*, *C.*

*beijerinckii*, *C. thermocellum*), as well as facultative anaerobes: *E. coli*, *Citrobacter* or bacteria from the *Enterobacteriaceae* family (*E. cloacae*, *E. aerogenes*), (Lee et al., 2011; Ntaikou et al., 2010). The process can take place in the presence of a pure or mixed culture, and the choice of a particular microorganism must be done adjusting to their specific requirements (Ntaikou et al., 2010; Wang and Wan, 2009). Each of these culture types has its own pros and cons. A pure culture is characterised by high selectivity, yielding higher hydrogen production efficiency with fewer byproducts. What is more, by changing growth conditions, it is easier than in mixed culture to manipulate the metabolism of microorganisms. However, such a pure culture is susceptible to impurities, which requires aseptic environment and increases the overall cost of the process (Ntaikou et al., 2010).

From the engineering standpoint, it is more desirable to deal with a process which uses a mixed culture coming from soil, compost or digester sludge (Howkes et al., 2007; Kleerebezem and van Loosdrecht, 2007; van Ginkel et al., 2001). Such a mixed culture, apart from hydrogen producing bacteria, also hosts microorganisms that, while not being hydrogen producers themselves, increase its production efficiency e.g. by creating biomass granules, using up oxygen, or decomposing complex organic substrates (Hung et al., 2011). A mixed culture of microorganisms can use a wider spectrum of substrates, and the cost of the process can be reduced since no sterilisation is necessary. However, the process also has some downsides. Such a mixture might contain microorganisms which do not produce hydrogen but compete for carbon sources (methanogens, homoacetogenes, lactic acid bacteria). In a mixed culture, domination of hydrogen-generating bacteria can be achieved by maintaining appropriate operation conditions during the process or by getting rid of the competing bacteria in a pretreatment step. For this reason, heat, oxygenation, and methods involving the use of acids, bases, chloroform and acetylene have been proposed (Akutsu et al., 2009; Argun et al., 2009; Hu and Chen, 2007; Kang et al., 2012; Ren et al., 2008; Valdez et al., 2006; Zhu and Beland, 2006). However, due to the different origins of inocula, substrate type and the specifics of the process conditions it is hard to determine unequivocally which of these methods is the most efficient way (Wang and Wan, 2009).

### 3.2. Substrates

The most efficient substrates for hydrogen production by means of dark fermentation are carbohydrates, such as glucose, sucrose and starch (Hawkes et al., 2007; Hallenbeck 2005; Lin et al., 2008), as well as arabinose (Abreu et al., 2012), xylose (Ngo et al., 2012) and glycerol (Seifert et al., 2009). However, pure substrates are very expensive, so in order for industrial-scale hydrogen production to be profitable, cheap waste products such as sewage sludge (Massanet-Nicolau et al., 2010), solid municipal waste (Dong et al., 2009), molasses (Li et al., 2007), and wastewater originating from biodiesel production (Han et al., 2012), olive oil production (Ntaikou et al., 2009) or palm oil production (Vijayaraghavan and Ahmad, 2006) have to be used. Over the past few years, first generation biofuels have been produced from energetic plants, such as oilseed rape or soybean. Nevertheless, their cultivation is controversial due to the fact that it requires high quality soil, which could be potentially used to grow food. This problem could be avoided by using second generation biofuels, e.g. hydrogen generated from plants such as sweet sorghum, switchgrass and miscanthus which can grow on land otherwise less desirable from an agricultural point of view, as well as from lignocellulosic residues produced by the wood processing industry (Magnusson et al., 2008). It is worth remembering, however, that such substrates contain complex polymers: cellulose, hemicellulose and lignin and therefore require pretreatment in order to dispose of lignin and change the structure of cellulose, which facilitates biodegradation of these waste products by cellulolytic microorganisms, but increases the costs of hydrogen production.

### 3.3. Hydrogen partial pressure

The bottleneck of dark fermentation is the partial pressure of hydrogen. An increase in hydrogen partial pressure shifts the metabolic activity of the bacteria towards synthesising more reduced products, thus decreasing the overall hydrogen production yield. Purging the bioreactor with nitrogen during the process increases efficiency because it allows to maintain the partial pressure of hydrogen at a low level and to dispose of carbon dioxide, which could potentially take part in acetogenesis, using up the generated hydrogen (Mizuno et al., 2000; Tanisho et al., 1998; Hawkes et al., 2007; Massanet-Nicolau et al., 2010). In a study conducted by Bastidas-Oyanedel et al. (2012) in headspace N<sub>2</sub>-flushing reactor, hydrogen production yield increased from 1 to 3.25 mol H<sub>2</sub>/mol<sub>glucose</sub> at pH 4.5 and N<sub>2</sub>-flushing of 58.4 l/d. The observed increase in hydrogen yield was explained to be thermodynamically controlled by low hydrogen partial pressure that affected lactate hydrogenase, NADH hydrogenase and homoacetogenesis reactions.

### 3.4. Temperature

Hydrogen production can take place in the presence of mesophilic bacteria (*Clostridium*, *Enterobacter*), thermophiles (*Caldicellulosiruptor*, *Thermoanaerobacterium*) or hyperthermophiles (*Thermotoga*). In a mixed culture, a change of the process temperature may affect the dominant bacteria culture. Karadag and Puhakka (2010) observed a change of the dominant culture from *Clostridium* in mesophilic conditions to *Thermoanaerobacterium* in thermophilic conditions. A comparison of the parameters suggests that hydrogen generation efficiency, as well as its fraction in biogas, was higher under thermophilic and hyperthermophilic conditions than that in mesophilic conditions (Shin et al., 2004; Valdez-Vazquez et al., 2005). This could be due to the fact that hyperthermophilic bacteria are less inhibited by the partial pressure of hydrogen and that due to high temperature fermentation is less susceptible to contamination with other cultures. This was confirmed by Shin et al. (2004) and Valdez-Vazquez et al. (2005), who did not notice any activity of methanogenes even though the inoculum was not pretreated. From an engineering point of view, a disadvantage of a thermophilic process is that a reactor must be heated up to desired temperature, and the fact that volumetric production rate of hydrogen is low, which would require an application of much bigger reactors than those used for mesophilic fermentation, and hence increase the costs (Hallenbeck, 2009).

### 3.5. Culture pH

pH is a key parameter to control hydrogen production, which affects the activity of hydrogenase (Dabrock et al., 1992), microbial communities structure, and their metabolism, modifying a spectrum of products (Guo et al., 2010; Ye et al., 2007). In another study, the optimum pH value during fermentative hydrogen production changed in a wide range from 3 to 9 (Gaddhamshetty et al., 2009; Lee et al., 2002), and strongly depended on the type of the substrate and inoculum (Wang and Wan, 2009). If food wastes were used as the substrate, the optimal pH value was about 5-6, whereas if the substrate were crop residues and animal manure, pH oscillated around 7 (Guo et al., 2010). Investigating *C. tyrobutyricum* ATCC 25755 (Zhu and Yand, 2004), it was observed that a change in pH affected the expression level of various enzymes. Enzymes responsible for creating butyrate and using up lactate were strongly expressed at pH 6.3, while other enzymes responsible for creating acetate and lactate at pH 5. However, this schema looks different for other organisms, such as *C. butyricum* CGS5 (Chen et al., 2005), for which an opposite relation was observed: the highest butyrate production rate took place at pH 5.5 and dropped as pH increased to 6.5. Therefore, while applying different microorganisms, it is necessary to check how pH affects the fermentation process (Cai et al., 2011).

#### 4. BIOREACTORS

Bioreactor configuration is of prime importance in hydrogen production process as it influences the microenvironment of the reactor, its established hydrodynamic behaviour, the prevailing microorganism population, and their contact with the substrate. For research purposes, batch reactors are used most frequently, because it is flexible and it is easy to operate. However, industrial-scale hydrogen production requires a continuous-flow bioreactor (Ntaikou et al., 2010).

The bioreactor type which is most frequently used is continuously stirred tank reactor (CSTR). Its construction is simple, it is easy to operate, has very effective stirring, and biomass is well suspended in a solution. It provides a good contact between the substrate and microorganisms, and a perfect exchange of mass (Ntaikou et al., 2010; Show et al., 2011). In CSTR, solids retention time (SRT) is the same as hydraulic time retention (HRT). Short HRT is favorable for hydrogen generation. However, using short HRT has both positive and negative aspects. On the one hand, it makes SRT short enough to protect a mixed culture from methanogen growth. On the other hand, if the HRT value is too small, biomass concentration in a bioreactor is limited, and often it may even be washed out from the reactor, which limits hydrogen generation rate and causes fluctuations in the whole functioning of a reactor (Ntaikou et al., 2010; Show et al., 2011).

During hydrogen production in CSTR reactors, bacteria can suddenly flocculate and create granules. This phenomenon is promoted by the presence of divalent cations and an increase in carbohydrate concentration in extracellular polymeric substance (EPS) (Jung et al., 2011 a). Moreover, Zhang et al. (2007) claim that a fast creation of granules had to be preceded by incubation in pH 2 for one day, which improved the physicochemical properties of the surface, favoring microbiological granulation, and facilitated biomass retention. Its content increased over 30-fold compared to the amount of biomass in CSTR reactor without granulation, which resulted in an increase of hydrogen generation rate to 3.2 l H<sub>2</sub>/l h (Zhang et al., 2007). Another example of a reactor with biomass retention is the membrane bioreactor (MBR). There are two types of such reactors: external cross-flow type and submerge type (Jung et al., 2011 a). The advantages of MBRs include higher biomass concentration in the bioreactor, hence a greater usage of organic substrate, a smaller reactor volume due to a higher substrate consumption rate, reduced production of excess sludge due to biomass decay in the reactor, and a lack of microorganisms in the effluent due to their total retention by the membrane (Oh et al., 2004). Lee et al. (2010) concluded, that in a submerged membrane reactor SRT was a key factor deciding about a stable hydrogen production process. In a reactor working on glucose under HRT of 9h, hydrogen production rate increased from 3.9 to 5.8 l/l d as SRT increased from 2 to 12.5 d. The hydrogen production yield changed in the opposite direction and reached the maximum of 1.19 mol H<sub>2</sub>/mol-glucose at the SRT of 2 d. An overly high SRT value (90 d) caused a drop in both the rate and efficiency of hydrogen production (Lee et al., 2010). A similar trend was also observed in previous research on an external cross-flow membrane bioreactor (Oh et al. 2004), where excessive biomass concentration increased substrate consumption, resulting in a decrease in hydrogen generation rate. Moreover, lower volatile suspended solids/total suspended solids were observed, as well as the metabolic pathway shift to lactate, and accumulation of extracellular polymeric substance (EPS) (Oh et al., 2004). EPS accumulation is the reason for the fouling of the membrane and it is one of the main reasons limiting the use of such reactors in biological processes (Lee et al., 2008a; Lee et al., 2010; Zheng et al., 2010).

An alternative to CSTR for continuous hydrogen production are upflow packed-bed reactors (PBR), in which the medium, e.g. wastewater, enters at the bottom and exits from the top. In such a reactor, biomass is immobilised either in granules or in biofilms or entrapped in packed media (Kothari et al., 2012). The support material included: sponge, granular activated carbon (GAC), expanded clay, polyethylene-octane elastomer, ceramic ball, alginate gel (Show et al., 2011). Compared to CSTR, PBR has higher mass transfer resistance, resulting in a lower substrate conversion and hydrogen production

rates (Show et al., 2011). However, Keskin et al. (2012), who compared hydrogen production from sucrose in a bioreactor filled with ceramic balls and CSTR with suspended cells, observed that in an 8 times smaller bioreactor with immobilised microorganisms at optimal HRT (3 h) the hydrogen production yield and rate were both higher (363 ml H<sub>2</sub>/g sucrose, 2.7 l H<sub>2</sub>/l d) than those in CSTR (87 ml H<sub>2</sub>/g sucrose, 0.5 l H<sub>2</sub>/l d, HRT 24 h). What is more, a packed-bed reactor was more resistant to biomass washing out observed in CSTR at low HRT (Keskin et al., 2012).

In a fluidised-bed reactor (FBR), gas or liquid passes through accumulated solid matter, causing its fluidisation. Such reactors are often characterised by good mixing, as well as high hydrogen production efficiency at low HRT and high biomass concentration. The methods for immobilising bacteria are similar to those used in packed-bed reactors. The kind of support material and microorganism immobilisation type has an important influence on hydrogen production parameters. It was confirmed in a study on glucose conducted by Zhang et al. (2008) and Barros et al. (2010). Zhang et al. (2008) used microorganisms immobilised on GAC or in a granulated form, at the same HRT (0.25 h) and similar VSS (35 g/l), achieving a comparable yield of 1.7 mol H<sub>2</sub>/mol glucose in both reactors, but the hydrogen generation rate was by 15% higher in GAC bioreactor, equalling 7.6 l H<sub>2</sub>/l h (Zhang et al., 2008). Barros et al. (2010), by using thermally pre-treated anaerobic sludge and polystyrene and expanded clay as the support material, at HRT 2h, achieved higher efficiency and hydrogen production rate of 2.59 mol H<sub>2</sub>/mol glucose and 1.21 l H<sub>2</sub>/l h respectively, in a bioreactor containing expanded clay. This was probably due to better surface characteristics of the expanded clay material for biomass attachment, which allowed to obtain a higher concentration of immobilised biomass on this material (1.1 mg TVS/g expanded clay vs. 0.805 mg TVS/g polystyrene) (Barros et al., 2010).

Hydrogen generation was also performed in up-flow anaerobic sludge blanket (UASB) reactor, used hitherto in methane production. This kind of bioreactor contains a gas/ liquid/solid separator on its top, where microbiological granules are formed. Such active biomass sediments easily, creating a thick biomass blanket zone at the bottom. The process in such a reactor proceeds effectively and with high stability (Hawkes et al., 2007; Jung et al., 2011a). For instance, the maximum hydrogen production efficiency and rate, with sucrose as the substrate, equaled 1.33 mol H<sub>2</sub>/mol hexose i 0.1 l H<sub>2</sub>/l h respectively (Wang et al., 2007). However, a disadvantage of UASB is its long start-up period i.e. the time necessary to form large enough granules, which in Wang's research was about 5 months. According to Jung et al. (2011b), the granulation rate can be increased by means of a two-stage process. Initially, the process took place in CSTR, after which the mixed liquid was moved to UASB as a seeding source. This strategy shortened the start-up period over 7 times (Jung et al., 2011b).

## 5. PROSPECTS

Hydrogen biotechnologies, in most cases, are not developed enough in order to put them to practical use. In 2009, in techno-economic analysis of biohydrogen production undertaken as a part of the EERE Hydrogen Production Program, the costs of generating 10000 kg/d of hydrogen by various methods were assessed (James et al., 2009). It was concluded that dark fermentation based on lignocellulose substrates can allow for production of 940 cm<sup>3</sup> H<sub>2</sub>/dm<sup>3</sup> h for 4.33 \$/kg. Given its high cost, this method could be competitive with the standard one, i.e. steam reforming of methane. However, for the costs to be realistic, a significant increase in both the rate and efficiency of fermentation processes needs to take place (James et al., 2009, Brentner et al., 2010). There is a need for further optimisation of the process using complex substances. Research has to be directed towards redirection and/or reconstruction of cellular metabolisms for example, by the elimination of enzymes and carbon pathways interfering or competing with H<sub>2</sub> production or the incorporation of non-native metabolic pathways leading to hydrogen production (Oh et al., 2011).

Another possibility is a development of integrated systems, in which hydrogen production by means of fermentation would be coupled to the next process. Organic compounds from wastewater after fermentation would be converted to methane, hydrogen of electrical energy (Hallenbeck and Ghosh, 2009). Such systems are already being tested in a pilot scale, for instance the coupling of hydrogen and methane fermentation of kitchen waste has allowed to remove 80 % of the organic matter (COD) and obtain 5.4 m<sup>3</sup>/m<sup>3</sup> d of hydrogen and 6.1 m<sup>3</sup>/m<sup>3</sup> d of methane (Ueno et al., 2007). Another process in which effluent from fermentation can be used is photofermentation, during which photosynthetic bacteria convert acids present in wastewater into hydrogen and carbon dioxide. This strategy increases the overall efficiency of hydrogen generation and significantly decreases the amount of organic substances (Chen et al., 2008; Zong et al., 2009; Yang et al., 2010). Electrical energy can be obtained in microbiological fuel cells (MFC) or in microbiological electrolysis cells (MEC), in which organic compounds that occur in post-fermentation wastewater are used (Logan, 2010; Sharma and Li, 2010). However, despite some encouraging results, each approach is still faced with many challenges that need to be addressed in future research before they can be fully and successfully implemented (Hallenbeck, 2009).

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