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Production of hydrogen from biomass and its separation using membrane technology

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Abstract:

Hydrogen is an important raw material for chemical industry and feasible renewable energy carrier that could replace fossil fuels. However, the specie seldom exists in a form of pure H_2. Therefore, to obtain hydrogen in volumes suitable to be used as a raw material it is necesary to decompose hydrogen-rich compounds. The carbohydrate-rich biomass can be an important source of hydrogen by applying the process of dark fermentation. In this paper potential ways of hydrogen production from organic wastes (biomass) by means of dark fermentation are reviewed and discussed. The bacteria used for dark fermentation are enlisted, characterized and compared. The pretreatment processes and various reactor designs are analyzed and discussed. The hydrogen separation by membrane method (which can provide the most pure hydrogen) are presented.

The paper describes recent achievements in optimizing parameters, conditions and reactors used to industrialize dark fermentation.

Keywords: dark fermentation, hydrogen, inoculum pretreatments, membrane separation

1.Background

Dark fermentation is a branch of science and technology which is developing very rapidly in every step of the process different substrates [1,2,3,5,8], including crop residues (such as corn [2, 3], bagasse[3,4], carrots [5], Jerusalem artichoke roots [5], maize flour [5], oats [5]), potatoes [1, 5], sugarbeet residues [2,6], wheat flour [7], rapeseed oil cakes [5], sunflower oil cakes [5], grape marc[8], vegetable waste from restaurants [8,9], fruit peels (orange peels and banana peels) [8], animal waste e.g. cow manure [7], chicken meat [8], fish residues [5,8], food residues like kitchen waste [5,7,8,10,], sewage wastes [1, 2, 5, 8, 11], and other biodegradation methods leading to hydrogen production [10]. Sambusti et al. [12] and Saiffudin et al. [13] reviewed dark fermentation taking into account one kind of substrate i.e. algae. Ghimire et al. [14] compared different substrates and parameters. Bundhoo et al. [15] and Wong et al. [16] analyzed role of pretreatments and parameters effecting the process. Elsharnouby et al [17] analyzed bacterial monocultures used for dark fermentation.

This review summarizes the role of substrates, bacteria and pretreatments, including parameters and reactors. In the article all the earlier steps and design of the dark fermentation process mentioned above are analyzed. Additionally in the article membrane separation methods are discussed.

Standard dark fermentation is an anaerobic process, which leads to the decomposition of sugar molecules (usually hexoses) into low-weight organic-acid, hydrogen and carbon dioxide. Hexoses and/or pentoses often originate from hydrolysis of higher carbohydrates such as starch, molasses and cellulose [15]. The great interest in dark fermentation based on different types of carbohydrates is generated because of the widespread availability of carbohydrate-rich materials (e.g. paper, wood, grass straws) with high hydrogen content and the low number of inhibiting byproducts that can occur during the process, together with the low amount of energy needed for bacteria to digest glucose.

According to Hallenbeck[19] and Gottshalk [20] dark fermentation can be a one-stage process, when the substrate contains simple sugars. Then, the general route of dark fermentation in the presence of water (for example, glucose or sucrose) is as follows [4, 5] as in equation:

$$C_nH_{2n}O_n \rightarrow C_mH_{2m-1}COOH+zCO_2+yH_2 ,$$

where: n = m+z = 5,6, 12, ...; y=0.5(n-m), y = 2 or 4; z=n-m-1.

Extended dark fermentation includes other biomaterials used successfully in anaerobic digestion, like fats, proteins, in addition to pure carbohydrates [14,22–25]. In the case of dark fermentation of substrates with high protein content the process can be disrupted due to high nitrogen and resulting high ammonia concentration inhibiting hydrogen generation [14,22], however, due to Alibardi et al. [26], proteins does not influence on dark fermentation process. Fatty acids are substrates with high potential for dark fermentation and high efficiency (hydrogen yield for sugars is around 0.33 but 0.38 for glycerol) [10,25,27,28].

Dark fermentation is related to methane fermentation, but the standard process is limited to hydrolysis and acidogenesis. Hydrogen production is optimized during acidogenesis under low pH conditions. Processes leading to methanogenesis are at least partly inhibited. In the case of dark fermentation process led by acidogenic bacteria (like Clostridium) methanogenic processes can be inhibited by special pretreatment of inoculum. Extended fermentation may rely on more stages, i.e. hydrolysis, acidogenesis and acetogenesis, but again it obstructs methanogenesis.

2. Bacteria promoting dark fermentation

Anaerobic microorganisms generate hydrogen using hydrogenase enzymes. Anaerobic bacteria produce hydrogen as by-product of their metabolism. The presence of hydrogenase enzymes was proven in 1931 in Escherichia coli. Hydrogenases are enzymes that stimulate production and recycling of hydrogen in bacteria[20]. Anaerobic bacteria produce hydrogen as a by-product of their metabolism. The most common anaerobic bacteria enzymes are: [Fe]-hydrogenase, [NiFe]-hydrogenase, [NiFeSe]-hydrogenase[30]. [Fe]-hydrogenase catalyses generation of hydrogen, while [NiFe]–hydrogenase uptakes generated hydrogen, and [NiFeSe]-hydrogenase is bidirectional. [NiFe]-hydrogenase is 100 fold less active than [Fe]-hydrogenase, therefore more generated hydrogen is excreted from the organism than is adsorbed back[20]. Hallenbeck pointed out that hydrogen can be generated by both: [Fe]-hydrogenase and [NiFe]-hydrogenase[15]. Morra et al.[31] reported existence of [FeFe]-hydrogenase enzyme in strict and facultative bacteria.

Dark fermentation can be stimulated by anaerobic bacteria of several different phyla, families, genus and species, belonging to Gram-positive or Gram negative groups. According

to Zajic et al.[18] there are several bacteria that produce hydrogen. Bacteria producing hydrogen are from a group of endospore-forming rods Bacillaceae (genuses Clostridum, Bacillus), Gram negative facultatively anaerobic rods (Enterobacteria, Vibrionaceae) and cocci (Veillonellaceae) [32], Gram positive cocci (Micrococcaceae), Peptococcaceae, Gram positive asporogenous rod-shaped bacteria (lactobacillae). Unfortunately, the majority of these bacteria produce hydrogen in amounts considered unsuitable for use in full-scale dark fermentation plants. Hydrogen is produced most efficiently by species of Clostridium, Bacillus, Enterobacter, and some thermophilic bacteria like Thermocellum and Thermatoga.

The role of these bacteria, strict bacteria (Clostridium) and facultative bacteria (Enterobacter, Bacillus) will be described below.

(a) **Clostridium**

One of the most relevant and the most efficient hydrogen producing groups of bacteria is Clostridium. An important feature of Clostridium is its ability to form protective spores. The protective spores allow surviving harsh conditions, like extreme temperature, low or high pH and chemical agents[33]. Another characteristic of clostridia is lack of cytochrome [34]. Therefore, inoculums containing Clostridium can be pretreated by means of heat, determined pH or chemicals for increase of the hydrogen production rate and to remove other bacteria. Hydrogen producing Clostridium are: Clostridium acetobutylicum, Clostridium botulinum, Clostridium butylicum, Clostridium butyricum, Clostridium cellobioparum, Clostridium cellulosolvens, Clostridium dissolvens Clostridium fossicularum, Clostridium hydrogenicus, Clostridium kluyveri, Clostridium oedematis-maligni, Clostridium pasteurianum, Clostridium sporogenes, Clostridium tetani, Clostridium tetanomorphum, Clostridium thermocellum, Clostridium thermosaccharolyticum, Clostridium welchii, Clostridium werni. Among these bacteria, beside thermophilic and mesophilic, also psychrophilic species appear like Clostridium algidixylanolyticum[35]. The most efficient hydrogenic bacteria are Clostridium butylicum, Clostridium butyricum, Clostridum kluyveri and Clostridum pasteuranium [36]. A monoculture of Clostridium sp. can produce from 1.61-2.36 mol H_2 mol^{-1}glucose[36]. Clostridia belong to strict anaerobic bacteria, the most important bacteria in mixtures which task is to produce hydrogen with the highest possible efficiency. Despite the high yield of hydrogen production clostridium are very fragile to oxygen and to various form of substrate[28]. Some clostridium like Clostridium sp. strain No. 2 are able to convert glucose and xylose with similar efficiencies [37]. There are attempts to reduce oxygen sensitivity by using them in mixtures with other less air sensitive groups of bacteria termed facultative.

(b) Bacillus

Bacillus is another group of bacteria which like clostridium are made up of endospore forming rods. The most commonly used are Bacillus macerans (acetoethylicus), Bacillus cloacae (Enterobacter cloacae), Bacillus macerans, Bacillus polymyxa. Kumar et al. [27] isolated Bacillus licheniformis from cattle manure. The hydrogen yield of dark fermentation with Bacillus licheniformis was 0.37 mol H_2 mol^{-1} glucose in semi-continuous process and 1.1 mol hydrogen mol glucose in batch mode [28]. The hydrogen yields for Bacillus coagulants from carbohydrates like cellobiose (5.6 mol H_2 mol^{-1} cellobiose), L-arabinose (1.9 mol H_2 mol^{-1} L-arabinose), D-xylose (1.2 mol H_2 mol^{-1} D-xylose) [29] are higher than in the case of bacteria from the Enterobacter group (Citrobacter freundi, Enterobacter cloacae).

c)Enterobacter

According to Zajic et al.[18] the family of Enterobacterae includes bacteria from seven groups. The genuses are: Escherichia coli (Genus I), Citrobater intermedius (Genus II), Salmonella enteritidis (Genus III), Genus IV (Enterobacter (Aerobacter) aerogenes), Enterobacter sp, Aerobacter cloacae, Aerobacter indologenes).

Enterobacterae is a group of bacteria that grow anaerobically or aerobically depending on pH value. Enterobacterae are anaerobic bacteria of high air-resistivity. Although oxygen blocks the growth of bacteria it does not decrease hydrogen yield. Therefore, Enterobacter is often used in mixed cultures, which are more sensitive to oxygen. They are used more rarely in monoculture due to lower hydrogen yield than in the case of Clostridium. According to Yokoi et al [30] for the Enterobacter aerogenes strain HO-39 hydrogen yield depends on the kind of carbohydrate substrate, i.e. from 0.83 moles of hydrogen for a mole of lactose to 2.16 mole of hydrogen for a mole of maltose. More detailed analysis of hydrogen production rate and yield are presented in Table 1.

Table 1. Dependence of hydrogen production rate and yield from carbohydrate in case of Enterobacter aerogenes strain HO-39[31]

Carbohydrate	Hydrogen production rate (ml H_2 l^{-1}subsrate medium)	Hydrogen yield (mol H_2 mol^{-1} substrate)
Glucose	1.243	1.00
Galactose	1.181	0.95
Fructose	1.094	0.88
Mannose	1.218	0.98
Mannitol	2.066	1.68
Sucrose	1.237	1.89
Maltose	1.343	2.16
Lactose	0.514	0.83

Bacteria can produce hydrogen in the wide range of pH value from 4.00 to 7.8 [31]. Therefore, Enterobacter aerogenes in relation to other bacteria can be considered as insensitive to pH change [32]. Ren et al. [33] extended the experiments of Yokoi et al. [31] to ramnose, arabinose, mannose, xylose and galactose, Table 2.

Table 2 Dependence of hydrogen production rate and yield from carbohydrate in case of Enterobacter aerogenes [31, 33]

Carbohydrate	Hydrogen production rate (ml H_2 l^{-1}subsrate medium)	Hydrogen yield (mol H_2 mol^{-1} substrate)
Xylose	1.77	0.79
Galactose	2.35	1.26
Ramnose	1.25	0.56
Mannose	2.42	1.3
Arabinose	1.81	0.81

Productivity of hydrogen from pure compounds like sucrose and glucose in the case of Enterobacter cloacae is higher than for Bacillus or Citrobacter. Hydrogen yields from sucrose

are: 5.6 mol H_2 mol^{-1} sucrose, and 2.8 mol H_2 mol^{-1} of glucose[35]. Mandal et al.[36] obtained a pure culture of Enterobacter cloacae using malt yeasts, and glucose mixture substrate of hydrogen yield 3.9 mol H_2 mol^{-1} glucose.

In the case of Citrobacter intermedium hydrogen yield is 1 mol H_2 mol^{-1} of glucose [18]. Hydrogen yields for Citrobacter freundi was found to be equal 5.4 mol H_2 mol^{-1} sucrose and 2.4mol H_2 mol^{-1} glucose.

d) other bacteria

A special group of bacteria is hydrogenic bacteria i.e. a genetically modified form of previously mentioned bacteria. Modification of bacteria is aiming to reduce or remove the possibility of hydrogenase uptake that leads to the recycling of hydrogen generated earlier, and to optimize the activity of hydrogenase. There are eighty types of hydrogenases[37]. The gene responsible for hydrogenase in Enterobacter cloacae was isolated and then transferred to non hydrogen producing Escherichia coli BL-21. It is known that Enterobacter can produce up to 6 mol of hydrogen/mol of sucrose [49]. The modification of Escherichia coli genes help them to produce even up to 1 μM of H_2 per minute[20]. Another aim is to block other competitive reductases.

According to Fang et al. [39], the bacteria most frequently modified for hydrogen production are Escherichia coli, Clostridium, Citrobacter and Klebsiella.

2.1. Optimal temperature conditions for bacteria

Bacteria can be classified taking into account optimal temperature of culture growth as extra thermophilic, thermophilic, mesophilic and psychrophilic. Thermophilic bacteria can produce hydrogen in the range from 45-90°C but their optimum is usually from 55°C to 60°C. Mesophilic bacteria can work in temperature from 25°C to 45°C with an optimum range between 33°C -37°C. Psychrophilic bacteria sustain in low temperatures from 5°C -25°C with optimum usually between 20°C -25 °C.

Some extreme thermophiles like Thermotoga neapolitana or Caldicellulosiruptor saccharolyticus in pure cultures can produce hydrogen from potato starch with the yield from 2.5-3.8 mol H_2 mol^{-1} glucose[40]. The hydrogen productivity of psychrophilic bacteria is much smaller therefore dark fermentation using this type of bacteria is investigated very seldom. The psychrophilic bacteria can be an efficient method for hydrogen production in high mountainous and high latitude regions [35,52]. Dębowski et al. [23] obtained biogas containing 65.2-69.1% of hydrogen and production from 1587.47 - 3087.57 ml H_2 g^{-1} biomass using these bacteria.

2.2. Inoculum pretreatment method

Inoculum pretreatment is a way of preparing the culture of injected bacteria to special task. There are several pretreatment methods: thermal, acid/base, aeration, microwave, ultrasonication and chemical supplementation.

a) Thermal pretreatment.

Thermal pretreatment methods include: heat shock, sterilization, freezing and thawing. Heat shock method is a method of boiling or drying of inoculum. The pretreatment is often used for preparing mixed-culture systems for hydrogen production [53,54]. According to Zhu and Beland [55] temperature range for heat-shock method should be between 80°C and 104°C and

exposure time from 15 to 120 min. On the other hand, Akobi et al.[56] preheated inoculum for 70°C for 30 minutes. Mixed cultures of Clostridium are boiled to a temperature of 100°C for 15 min [45]. However, according to Kotay and Das [29] heat treatment for 20 min, in temperature 121°C decreased competitive microbial cultures to 2% after heat pretreatment. The heat-shock method disables hydrogenotrophic bacteria that uptake generated hydrogen and compile anaerobic bacteria like clostridium [46]. The non-spore forming methanogens should be removed from system after pretreatment [47]. In the case of clostridium species heat shock pretreatment leads most often to the butyric type of fermentation [48]. Logan et al. [49] used drying of mixed culture in samples thickness of 1 cm thick in an aluminum pan for 2 h at T=104°C. Then samples were sieved through a mesh (850 μm) and stored in bottles at a temperature of 4°C. Zhu et al. [46] states that methanogenesis inhibits hydrogenesis if methane content in biogas is above 2%. Boiling of clostridium reduces activity of uptake hydrogenase [62–64]. The method can be used for both mesophilic and thermophilic conditions Zhang and Shen[65] placed inoculum in cracked cereal baked for 2 h and then boil for 30 min. Mu et al. [48] used heat shock at temperature 102°C for 90 min for anaerobic sludge from wastewater fermentation obtaining a yield of 2 mole H_2/mole glucose.

Chaganti et al. [50] applied heat shock at 90°C for 30 min which achieved a hydrogen yield 2.84 mol/mole of glucose. Sterilization or pasteurization is a method that could be performed by twice heating of activated sludge for 20 min at a temperature of 80°C and then boiled anaerobically digested sludge for 15 min [24]. Palazzi et al. [51] used sterilization with autoclaving at 120°C for 20 min. For Hawkes et al.[52], a heat-shock of 100°C for 1 h is the most suitable in the case of agricultural soil used as an inoculum source. Lag time after heat treating depends on the origin of inoculum. For municipal sludge optimum lag time is 2 days while for microcrystalline cellulose is 4 days [36,69,70]. In the case of freezing and thawing method, inoculum is firstly frozen to -10°C and kept for 24 h and then thawed up to 30°C slowly over 6 hours. Kotay and Das [55] applied freezing of sample to -20°C for 6 h and then thawed up to 20°C for 6 h, obtaining a yield of 6.5 ml H_2 g^{-1} COD.

b) acid pretreatment

The acid pretreatment applies usually strong acids like acid chloride. The acidic pretreatment is performed at pH from 3.0 to 5.0 [32] for 24 h. Then pH is adjusted to the level of 7.0 by hydroxide solution like 0.1M NaOH [44,72]. Chaganti et al. [50] used 2.0 M HCl pretreatment at pH 3.0 and inoculum was incubated for 24 h at temperature 37°C. According to Ruggeri et al. [57] the acidic pretreatment led to hydrogen concentration increase between 50-70% in outflow. Methanogenesis does not occur or occurs in minimal amounts. Furthermore, acid pretreatment in the case of clostridium can lead to mixed acetic and butyric fermentation [58]. Acid pretreatments in particular improve conditions of dark fermentation with the use of Clostridium sp. [59]. Mu et al. [60] improved hydrogen yield from wastewater by applying the addition of up to 1.3 mol H_2 mol^{-1} glucose, in contrast to Ruggeri et al's experiment. [59] where 0.42 mol H_2 mol^{-1}glucose was added.

Chaganti et al.[50] achieved a hydrogen yield of 3 mol H_2 mol^{-1}glucose. According to Hu and Chen [21] acidic pretreatment reduces the methanogenic phase of clostridium to negligible amounts in the case of sewage sludge. Methanogenic yield of clostridium granules is reduced to 61 ml CH_4 g^{-1} glucose.

c) base pretreatment

Under base or alkali-pretreatment conditions, inoculum is kept at pH = 10-13 for 24[h][43]. The pH~10-13 is obtained by adding hydroxides usually 1.0-4.0M solution of sodium hydroxide. However Kim et al.[77] also used potassium hydroxide, magnesium hydroxide and calcium hydroxide. After pre-treatment pH is lowered to the level of pH =7.0 by addition of a strong acid such as 0.1M HCl [75]. Mu et al. [60] obtained a hydrogen yield of 0.48 mol H_2 mol^{-1} glucose from wastewater, lower than using acid or heat shock pre-treatment. The alkaline method leads to mixed butyric and acetic fermentation in similar ratios[78]. The inoculums, in order to start hydrogen production, need to be kept at pH from 5.0-5.5 by continuous addition of alkali to maintain stable pH [79]. Chaganti et al. [66] used base treatment with 3.0M NaOH to keep inoculums at pH=11 and then left 24 h for incubation at 37°C. The yield achieved was 2.8 mole of H_2 per mole of glucose. The alkali prestreatment inhibits methanogenic behavior of bacteria according to ref. [75].

d)aeration

Aeration can be used for anaerobic bacteria quite resistant to oxygen like Enterobacter. Yokoi et al. [19, 49] used 12 h aeration in temperature 30°C in the basal medium of glucose polypepton at pH 6.5. Palazzi et al. [67] used aerobic pretreatment in 37°C for 12 h in rotary shakers; later, the bacteria cells were moved to stationary phase. Bagley and Kramer [81] treated the sample with air for 1 h before placement in the reactor. Zhu and Beland [55] continued pretreatment for 0.5 h and obtained yield 4.7 mol H_2 mol^{-1}sucrose.

e)microwave

Guo et al. [82] prepared inoculum by heating the sample in a microwave reactor powered at 560 W for 2 min. and later obtained a yield of 11.04 ml H_2 g^{-1}COD [82]. Kotay and Das [40] used microwaves with power 600 W for 2 min. obtaining a yield of 8 ml H_2 g^{-1} COD.

f) ultrasonication

According to Hsia et al.[83] ultrasound pretreatment stresses with thermal and nonthermal (mechanical) and empty-cell effects like radiation pressure, radiation force, acoustic torque, acoustic streaming effect and cavitation. Thermal effect refers to micromassage causing tissues to generate ultrasonic efficacy and thus produce additional heat energy and also refers to the increased heat production by the biomass organisms after eating, owing to the metabolic energy cost of digestion [83]. Kim et al. [77] applied ultrasonication with a frequency of 42 kHz from 10 to 120 min. to a sample of inoculum. Approximately 18.4% of COD was converted to hydrogen. The effect of ultrasonic pretreatment was increased by earlier thermal pretreatment in (121˚C at pressure 1.5 atm for 30 min. and addition of 7g/l NaOH); 19.4% COD conversion was achieved [77]. Kotay and Das [40] used sonification at frequency 20 kHz and power 140W, interacting at 2 mm depth, under temperature 25°C. Hsia et al [83] obtained hydrogen production rate of 271 ml of H_2/h at 4 J energy for 15 minutes at frequency 0.5 MHz at starch concentration 30 g/l.

g) chemical supplementation

Ghimire et al. [22] and Zhu and Béland [55] proposed using BESA (sodium 2-bromoethane-sulfonic acid) method for inoculums pretreatment. BESA was added to kill methanogenic bacteria after pasteurization at 120°C for 30 min. [14]. The methanogenesis of 0.1 g cells of bacteria can be blocked by 0.01 mol of BESA. Zhu and Béland [55] proposed also an

iodopropane method in which diluted iodopropane in ethanol is added to sample with inoculum at room temperature for 30 min.

Chaganti et al. [66] proposed to add linoleic acid to inoculum. The inoculum was a mixed culture consisting: 26% Archea, 10% Bacteroidaceae, 12% Bacillaceae, 33% Clostridiaceae, 6% Enterobacteriaceae, 6% Geobacteriaceae and 5% Methylobacteriaceae. The inoculum was treated with 2000 mg of linoleic acid and then left in 37°C for 24 h. Then, inoculum was aligned to pH=5.5 using 1M HCl and 1M NaOH solutions. The obtained yield was 3.48 mol H_2 mol^{-1}glucose. According to Hu and Chen [33] chloroform pretreatment could be an efficient method for sludge in granular form; however according to Wang and Wan [54] it is less efficient than alkaline, aeration, and the thermal method. In case of culture of Clostridium, hydrogen production is more resistant to chloroform presence of higher concentration than methane production in both granule and sewage sludge. Therefore, in Clostridium culture the methane production can be blocked by adding chloroform in the range from 0.05-2.5% [33]. The addition of chloroform above 2.5 blocks both hydrogen and methane fermentative production [31]. The optimal amount is 0.1% of chloroform; hydrogen production was 180ml H_2 g^{-1}glucose in hydraulic retention time of 3 days [54]. The pretreatment method proposed by Nath et al. [84] based on keeping the sample in solution 1% v/v chloroform for 24 h at temperature 25°C.

h) Centrifuging

These methods apply centrifuging wash-out of bacteria cells as stress condition. Yokoyama et al. [85] used centrifuging of frequency 1500 rpm for 15 minutes for pretreatment of inoculum from cow waste slurry before applying thermophilic conditions in a batch system. Cigneroz-Perez et al. [86] performed the pretreatment using frequency 14000 rpm for 15 minutes. A pretreatment method at frequency 100 rpm for 30 minutes was applied in the case of wheat waste or powder [87–92]. In case of cheese whey powder Kargi et al. [93] used frequency 8000 rpm for 30 minutes. Yokoyama et al. [85] obtained the highest hydrogen production of 392 ml H_2 per litre of slurry. Perez-Pimienta et al. [69] obtained 176 ml H_2 per litre of organic wastes. Kargi et al. [93] obtained hydrogen productivity of 142 ml H_2 from one litre of cheese whey powder. In the case of wheat waste, hydrogen production was 77.375 ml per litre of wheat waste [95] and 223 ml H_2 from g of starch (from wheat powder) [96].

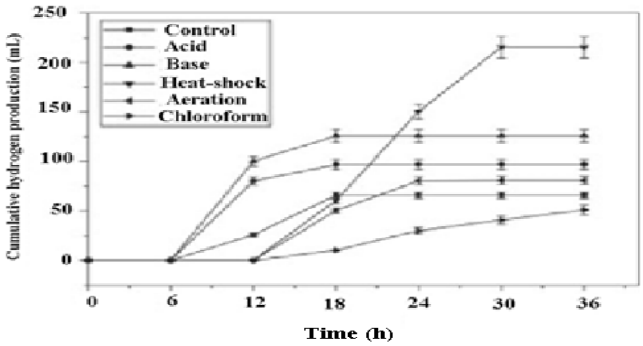


Fig. 1. Effect of pretreatment method of inoculum on cumulative hydrogen production[54]

i) Comparison of methods

According to Wang and Wan[54] selection of the most efficient method of pretreatment for hydrogen production depends on projected time of the preparation of inoculum. The cumulative hydrogen production rate as a function of pretreatment method duration is presented in Fig. 1.

As can be seen the most efficient method for the time lag between 6-22.6 h is the base pretreatment method. For process longer than 22.6 h the heat shock method is the most useful. Less efficient is the acid method. The heat shock method was less efficient than base pretreatment in pH~10.0 for 30 min or aerobic method. In the case of applying secondary batch recultivation basal pretreatment was the most efficient leading to 6.12 mol H_2 mol^{-1} sucrose. During alkaline pretreatment, fewer metabolites are produced than in a heat shock. In the case of mixed culture of *Enterobacter cloacae* IIT-BT 08, *Citrobacter freundii* IIT-BT L139 and *Bacillus coagulans* IIT-BT S1 (ratio 1:1:1) according to Kotay and Das [97] the most efficient pretreatments were: heat shock, microwave and base pretreatment. The heat shock pretreatment leads to a yield of 14 ml H_2 g^{-1} COD, while microwave and base pretreatment resulted in 8 ml H_2 g^{-1}COD. Ultrasonication and acid pretreatment were also quite efficient methods; in both cases yields were ca. 7 ml H_2 g^{-1} COD.

According to Zhu and Béland [55] the most efficient pretreatment method with one batch cultivation is that which applies iodopropane and BESA.

3. Reactors used in dark fermentation.

Reactor design is an important factor to ensure the process of dark fermentation can be controlled, by selection of process temperature, mixing speed, the surface of reactions and pH of the medium. A proper selection of reactor type enables the maintenance of suitable conditions for efficient production of hydrogen.

Reactors differ by type of the process (continuous, batch, -semi-continuous) and its phase multiplicity e.g. two-phase and multiphase reactors. The bioreactors can be singular or work in parallel or in series. Reactors in series allow high conversion of the substrate to be obtained, while the singular ones are cheaper and simpler in form. The continuous reactors include CSTR (continous stirred tank reactor), ANABR (Anaerobic Baffled Reactor), UASB (Upflow Anaerobic Sludge Blanket reactor), fluidized bed reactor, packed-bed reactor, and fixed-bed reactor [98]. The batch type includes: vials, fermenters and leaching-bed reactors [99,100]. A chemostat is an example of a semi-continuous type reactor [101,102]. Reactor are described and then compared in table 3.

a) *Batch type*

Batch type reactors are the primary option in most experiments of dark fermentation [5]. **An advantage of the batch process is its simplicity, high conversion–level of substrate and small pretreatment requirements**. A disadvantage of batch type reactors is generally low production rate. The most common material for construction of a batch type reactor is glass [103,104] A batch reactor for an anaerobic process is called ABR (Anaerobic Batch Reactor) or ASBR (Anaerobic Sequencing Batch Reactor) [72]. A specific kind of batch process were performed in serum bottles [37, 57] used for testing inoculums growth under different conditions. Logan et al. [105] obtained high level (23%) of conversions of glucose and sucrose into hydrogen, lower level (15%) from molasses, (0.5%) from lactate and (0.075%)

from cellulose. Shin and Han [106] designed a special type of batch reactors in a series (leaching bed reactors in rotation mode) for hydrogen production from food waste. The hydrogen yield of the whole series was 310 ml H_2 g^{-1}food waste. The hydrogen production rate was 1321.6 ml H_2 $l^{-1}h^{-1}$. In the case of glucose the yield was 1.04 mol H_2 g^{-1} glucose [72].

b) Semi-continuous reactors

The semi-continuous process can be used for preparation of bacterial cultures for continuous process that prevents wash-out in a continuous stage. The semi-continuous part includes intervals of feeding and digestion[14,107,108]. Generally hydrogen yield is lower than batch process but this type allows for better reaction control and is also used as preparation of bacteria in continous flow [109]. The semi-continuous reactors are semi-continuous drummer and chemostat. Oh et al. [59] applied in chemostat mixed cultures of Clostridium acidisoli CAC237756, Linmingia china AF481148, and Cytophaga sp. MDA2507AF238333 (Flexibacteraceae). The concentration of hydrogen in off-gas was 57-60%. In the feed was glucose of concentration 10 000 mgl^{-1}. The conversion of glucose to hydrogen for HRT 5 h was 20% at pH =5.5 [59].

Semi-fed process for swine manure fermentation was performed in 8 l tank with hot plate stirrer with 200 rpm [58]. Chen et al. [110] used the same type (4 l) reactor for investigation of culture kinetics. The semi –continuous mode can be used as the start-up stage of continuous processes like in Chen and Lin [111]. The semi-continuous process can be used for preparation of bacterial cultures for continuous process that prevents wash-out in a continuous stage. The semi-continuous part includes intervals for feeding and digestion.

c) Continuous process Continuous stirred tank reactors (CSTR's)

Advantages of CSTR are: high mass transfer due to mixing, simplicity of construction and operation. The CSTR process is limited by: low biomass concentration, cell retention in low dilution rate and risk of washout of cells at a high dilution rate [112]. Continuous stirred tank reactors (CSTR) are often used to investigate the influence of process conditions in the case of continuous process in lab scale like in Kim et al. [40, 62]. Ren et al. [46] developed a pilot plant based on CSTR reactors, where the hydrogen production rate from molasses was 201.4 ml H_2 l^{-1}molasses/h . A continuous process was designed also for fermentation of a xylose and glucose mixture by Taguchi et al. [37]. The continuous process provided a higher hydrogen yield than in the case of the batch process. In the case of pure xylose, Taguchi et al. [37] obtained a higher hydrogen production rate in continuous than in batch mode, while hydrogen yield was lower. Yokoi et al. [41] used the continuous dark fermentation stage during the hybrid process of dark and photofermentation. Inoculum is often prepared in a batch mode reactor and then transferred into a continuous mode reactor.

The hydrogen yield from continuous fermentation of starch using mixed cultures of Clostridium butyricum and Enterobacter aerogenes HO-39 was 2.9 mole of hydrogen per mole of glucose [80]. Hussy et al. [68] obtained a hydrogen yield of 1.3 mole of hydrogen per mole of glucose in the CSTR process of mixed cultures of Clostridium butyricum and Enterobacter aerogenes [68]. Besides, a high organic load-rate cannot be used [28]. Therefore, Wu et al. [114] modified CSTR by inserting anaerobic sludge immobilized by seeding with silicone. Such seeding enables granulation of sludge and increased biomass concentration to 35 g of biomass/l to be obtained. The highest hydrogen yield for Clostridium pasteuranium

was 1.93 mole of hydrogen/mol of hexose or 3.5 mol H_2/mol sucrose. The hydrogen production rate was l15.1 ml H_2 h^{-1} l^{-1} or 0.61 mol H_2 h^{-1} l^{-1} for initial concentration 40 g COD l^{-1} [114]. At an initial substrate concentration of 30 g COD l^{-1} the hydrogen production rate was 14.5 ml H_2 h^{-1} l^{-1} [114]. The specific hydrogen production rate was 0.439 l H_2 h^{-1} g^{-1} biomass [64]. The scheme is shown in Fig. 2.

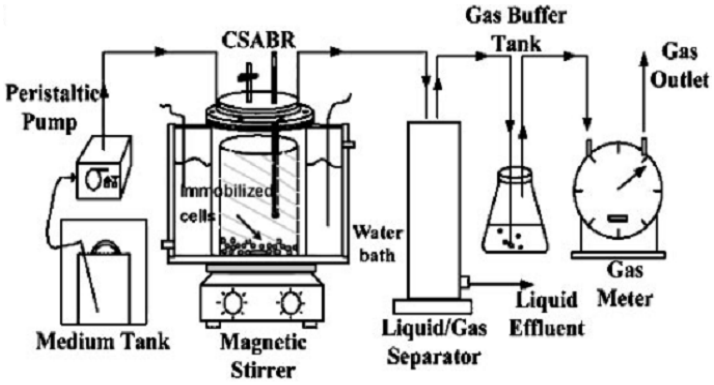


Fig. 2. Scheme of continuous stirred anaerobic bioreactor, after [64]

Wu et al. [115] used a CSTR reactor, while Ghimire et al. [22] a UASB reactor, as incubator for 8 h HRT of sludge for fluid-bed reactor. According to Hawkes et al. [36] optimal conditions for the continuous process of simple substrates are: pH= 5.5, working temperature 30°C and HRT 8 h – 12 h.

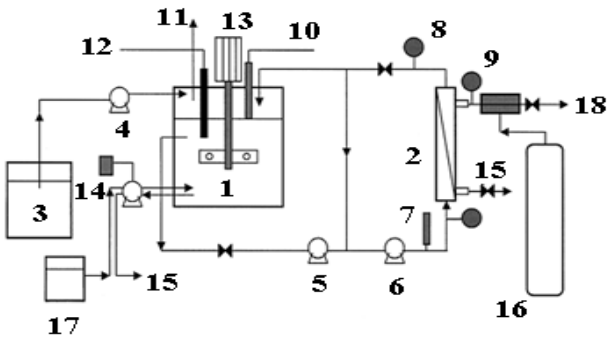


Fig. 3 Diagram of membrane bioreactor for hydrogen production. 1 - anaerobic reactor; 2 - cross-flow membrane; 3 – influent purged with nitrogen; 4 - feed pump; 5 - recirculation pump; 6 – high recirculation pump; 7 - flow meter; 8 - manometer measuring pressure at inlet, outlet and permeate side; 9 – backpulsing every 10 s to avoid fouling; 10 - level controller; 11 - gas monitor; 12 - pH controller; 13 - motor; 14 - timer;15 - waste; 16 - nitrogen gas; 17 - medium w/o organics; 18 - effluent after [59].

d) Membrane bioreactor

Membrane reactors increase conversion by separation of products but only in short detention times not longer than 3.3 h HRT. In this condition internal fouling is minimal[59]. A membrane bioreactor is a semi-continuous bioreactor with applied cross-flow membrane module [59] - see Fig. 3. A ceramic-alumina membrane module of tubular type keeps the biomass in the reactor [35]. The applied membrane facilitates increasing conversion of glucose to hydrogen from 20% to 38% [59]. The dilution rate was decreased while sludge retention time was increased. KOH was used as a pH controller. The recycle-loop flow-rate was 378 l h^{-1} with cross-flow velocity 2.8 m/s [59]. Before process membranes were cleaned by rinsing with 1% nitric acid for 2 h, 2% of NaOCl with water for 2 h. Pore sizes of membrane were 0.2 μm, 0.5 μm and 0.9 μm [35]. Membrane surface was 55 cm^2[35].

e) Fluidized bed reactor

Fluidized bed reactor allows the use of high volume fraction of biomass without risk of attrition like in CSTR. Besides, bubble column like reactor allows for high mass transfer. Wu et al. [65, 67] applied fluidized bed reactors with acrylic latex plus silicone to immobilize sludge, see Fig. 4. The bed of sludge consisted of particles of 3.0-4.0 mm formed from a $CaCl_2$ mixture of alginate sodium with 75% (v/v) acrylic latex/silicon. Additionally, seed from municipal sludge was supported by alginate gel. The use of sewage sludge as a seed in fluidized bed and optimal concentration of sucrose 17.8 g l^{-1}, provided a hydrogen yield of 1.34 mol H_2 mol^{-1}hexose[116]. Relevant parts of bed reactors (fluidized, packed or fixed) are carrier materials that keeps bacteria in the form of biofilms. According to Barca et al.[98] carrier material diameters range from 0.2 and 4 mm, and their density between 1.05 and 1.50 g/cm^3.

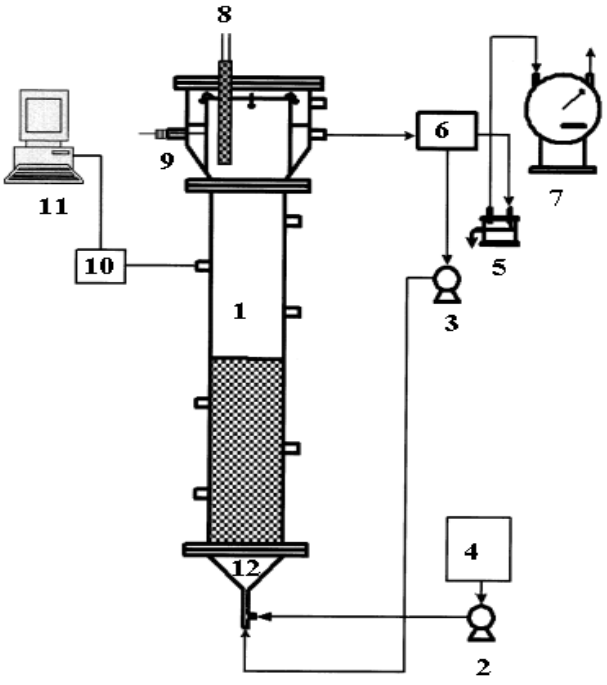


Fig. 4. Fluidized bed column of diameter 8 cm, static bed height 40 cm and column height 120 cm, total working volume 10l (1); 2 - pump for substrate, 3 - recycle pump,

4 - substrate tank, 5 - gas-liquid separator, 6 - buffer tank, 7 - gas meter, 8 - heating coil, 9 - thermal couple, 10 - PF acquisition, 11 - PC analysis system, 12 - liquid distributor[116].

f) Packed-bed reactor.

Packed-bed keeps biomass in the reactor, preventing its washout like in CSTR. An example of a scheme of setup with packed-bed reactor is shown in Fig. 5. Various packing materials were used: glass beads, activated carbon, ceramic fittings and polymeric (for example polyethylene)[67,98].

In the case of these reactors Barca et al. [98] report that carrier material diameters are from 1.5mm to 25 mm and density from less than 0.5 up to 2 g/cm^3. The ratio between carrier particle diameter and reactor vessel diameter should be less than 0.1 [98,117]. Infra particle porosity (an important parameter of the reactors) depends on the material. Barca et al. [98] pointed out that increasing of porosity results in increased adhesion of hydrogen, while more rough surface better protects biofilms from shear stresses. When activated, carbon porosity is in the range 1100–1350 m^2/g and for polyethylene pellets 12 cm^2/g [118,119]. The plugging of the packing by substrates can be prevented by designing proper configuration of packing. According to Kumar and Das [120] in dark fermentation the most efficient configuration of packing material (from among tubular, rhomboidal or tapered) is rhomboidal. The packing material was designed using a lignocellulosic matrix. At the optimal glucose concentration of 10 g l^{-1}: hydrogen yield was 2.04 mol H_2 mol^{-1} glucose, the specific hydrogen production rate was 6.85 l H_2 h^{-1} g^{-1} biomass and volumetric hydrogen rate was 1.85 l H_2 h^{-1} l^{-1} substrate. The highest hydrogen production rate was 75.6 mmol H_2 l^{-1}h^{-1}.

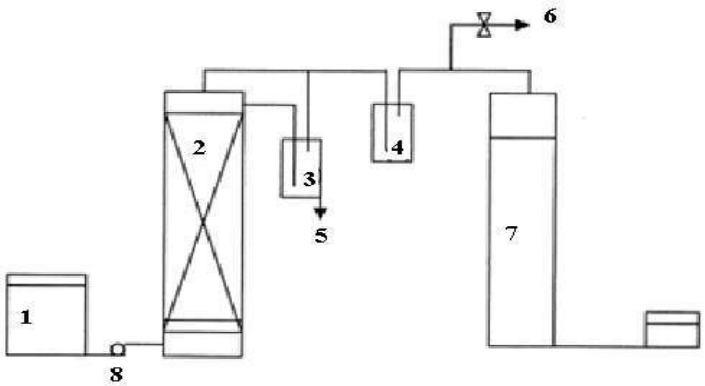


Fig. 5 Scheme of the diagram with packed-bed reactor with rhomboidal packing material: 1 - feed tank, 2 - packed bed reactor, 3 - liquid trap, 4 - CO_2 absorber, 5 - effluent, 7 - gas collector, 8 - peristaltic pumps [67]

Palazzi et al. [67] applied a packed column for dark fermentation of starch with Enterobacter aerogenes. Packing to immobilize bacteria was composed of spongy particles and glass beads. Another form of packing was composed of coir magnetite nanoparticles [121,122]. Sponge particles of dimensions 5×5×2 mm^3 were obtained after sterilization of sponge. Applying glass beads of diameter 7 mm with spongy particles lowered uniform residence time

distribution in the bed. If the flow rate was low (4 cm^3h^{-1}) and residence time was high (100 h), the reaction was shifted towards butane-2,3-diol, and the hydrogen production rate was 1.3 mmol H_2 h^{-1}. Selectivity of hydrogen was increased when the flow rate was increasing, from 4 to 40 cm^3h^{-1}, and residence time decreased, e.g. ~10 h. The hydrogen production rate at flow rate 40 cm^3h^{-1} was found to be 4.06 mmol H_2 h^{-1}, but its yield decreased from 3.02 mmol H_2 mol^{-1}glucose to 1.54 mmol H_2 mol^{-1}glucose [67]. Barca et al. [123] used glass beads of 4mm in diameter and porosity 0.38 as packing for biofilm of Clostridium acetylobutylicum and Desulfibrio vulgaris. They obtained a hydrogen yield 1.34 mol H_2/mol glucose and hydrogen production 0.097 l H_2 /h l glucose.

g) *Fixed-bed reactor*

In a fixed-bed reactor a biofilm typically replaces the catalytic layer. The advantages are generally lower pressure drop together with simple and robust construction. Other advantages mentioned by Contreras–Davila et al. [124] include higher magnitudes of volumetric mass transfer coefficients. The disadvantage of fixed-bed reactor is limited surface area [125].

Chang et al.[126] designed fixed bed reactors with support matrices, such as expanded clay and activated carbon. Anzolar-Rojas et al. [127] used as a support recycled polyethylene cylinder-shaped particles of diameters from 7.1mm and 17.5mm and length 30mm. For expanded clay working volume 0.3 l, hydrogen volumetric production rate was 0.415 l H_2 h^{-1} l^{-1} sucrose at 20 g COD l^{-1}, specific hydrogen production rate was 0.0965 l H_2 $h^{-1}g^{-1}$ biomass [110].

Wu et al. [116] designed a fixed bed reactor for dark fermentation from sucrose, glucose, and fructose. Polyethylene octane elastomer was used as a sludge and catalyst. The hydrogen production rate increased by increasing up-flow velocity to 0.91 cm s^{-1} [116]. Gomes et al. [128] designed a multiple-tube fixed bed reactor with PVC tube avoiding washing out. The hydrogen production rate was 0,061 l H_2 h^{-1}[128].

(h) Upflow Anaerobic Sludge Blanket (UASB) *reactor*

UASB reactors are characterized by conversion of biomass in large quantities and using high organic load. However, according to Lee et al. [129] the main disadvantage of the UASB reactor is its sensitivity to the channeling effect causing loss of contact with the substrate and the bed. The reactor with mixing of 120 rpm was used by Mu et al. [60].

The expanded granular sludge bed (EGSB) reactor is a special type of UASB. EGSB is characterized by a larger height to diameter ratio than that of UASB and so recirculation of effluent velocity causing higher up-flow velocity [129]. Hernandez et al.[130] used as a support material recycled tire rubber. Kisielewska et al. [131] obtained from whey permeate 0.29 l H_2/h and yield 4.55 mol H_2/kg COD. Sui et al. [132] used SiC support obtaining hydrogen production rate 0.22 l H_2 /h and hydrogen yield 0.93 mol H_2/mol of glucose. Rosa et al.[133] from cheese whey obtained 1.33 mol mol H_2/mol of lactose and hydrogen production rate 0.51 l H_2/h.

(i) Carrier-induced granular sludge bed (CIGSB)

Lee et al. [129,134] designed a CIGSB reactor **to improve mixing properties** by applying a different variation of agitation system (physical, mechanical) and a different height to diameter ratio to the reactor. Comparing height to diameter ratios of 4, 8 and 12, the ratio 8

results in the highest hydrogen generation from sucrose in wastewater. The hydrogen production rate was 9.3 l H_2 h^{-1} l^{-1} of sucrose and a maximum H_2 yield of 4.02 mol H_2 mol^{-1} substrate.

Table 3 Comparison of techno-economical aspects of reactors

Reactors	Technical aspects	Max. yield	Economical aspects	Ref.
Batch	Simple and facile system operation, high yield	4708 ml H_2 /g glucose	The hydrogen production potential is too low for industrial scale	[135]
Semi-continuous	Necessary bacteria preparation for continuous regime	460 ml H_2/g glucose	The hydrogen production potential is too low for industrial scale	[136]
CSTR	High risk of bacteria washout; mixing allows intimate contact between substrate and biomass; efficient pH and temperature control	63 ml H_2/g glucose	The simple form, cheap	[137, 138]
Membrane	HRT shorter than 3.3 h; much lower conversion than in CSTR	116 ml H_2/g glucose	Costly membrane exchange due to possible fouling.	[59]
Fluidized-bed	Risk of biomass over-accumulation	12 ml H_2/g glucose	High complexity of the system and the high energy costs	[6]
Packed-bed	Risk of biomass over-accumulation; limited mixing	125 ml H_2/g substrate	Complexity is less than in fluidized bed but still high	[123, 139]
Fixed-bed	Risk of biomass over-accumulation	11 ml H_2/g sucrose	High pressure drop can make process hard to implement	[140]
UASB	High treatment efficiency, low and stable HRT; no granulation of biomass is observed; more tolerant to fluctuation of process parameters than CSTR	263 ml H_2/g glucose	High pressure drop can make process hard to implement; long term production stability possible	[114, 141–143]
CIGSB	Poor efficiency of mass transfer, risk of bacteria washout.	179 ml H_2/g sucrose	High pressure drop can make process hard to implement	[134]

(j) Multi –stage process

In the case of two-stage dark-fermentation process, the feed preparation and hydrolysis are kept as a continuous process e.g. in a continuous stirred tank reactor but the second stage proceeds e.g. in the batch periodic anaerobic baffled reactor (PABR) [144]. At the second stage sugars are converted to hydrogen, carbon dioxide and organic acids. The hydrogen production rate was 7.53 l H_2 per day with removal of 95% of COD (cheese whey) [144].

Another type of hybrid batch and continuous stirred tank reactor with sludge was used by Wu and Chang [72]. Sludge was immobilized by cells from activated carbon and PMMA (Poly(methylmethacrylate)). The substrate of hydrogen was sucrose from wastewater. 90% of sucrose was converted [72]. The maximum production rate was 1800.4 ml H_2 l^{-1} h^{-1} and maximum H_2 yield 2.25 mol H_2 mol^{-1} substrate [72].

4. Influence of process parameters on hydrogen yield in dark fermentation

In order to optimize the process of dark fermentation (in relation to the highest hydrogen production rate) one should aim at: increasing efficiency of the Fe-hydrogenase and usually

inhibiting of the NiFe-hydrogenase as well as obtaining optimal conditions for bacterial culture growth.

It is well known that the efficiency of dark fermentation is influenced by process parameters such as: feed type, temperature, partial pressure, pH and presence of metal ions. The importance of a particular factor depends on the reactor type and feed.

4.1. Feed type

According to Hallenbeck [145] and Gomez et al. [6] theoretical maximum yield of hydrogen from hexoses (including glucose) in dark fermentation is 32%. Similar yields were obtained for hydrogen production from pentoses (30-33%) [27]. In the case of glycerol the hydrogen yield in dark fermentation is 38% [25].

Bartacek et al. [28] and Woodward et al. [146] pointed out that there are three thermodynamically possible dark fermentation pathways from hexoses: acetate equation (1), butyrate equation (2) and acetate-ethanol equation (3):

$$C_6H_{12}O_6 + 4H_2O \rightarrow 2CH_3COO^- + 2HCO_3^- + 2CO_2 + 4H_2 + 2H^+, \quad \Delta G^0 = -48 \text{ kJ mol}^{-1}, \quad (1)$$

$$C_6H_{12}O_6 + 2H_2O \rightarrow CH_3CH_2CH_2COO + 2HCO_3^- + 2CO_2 + 2H_2 + 3H^+, \quad \Delta G^0 = -137 \text{ kJ mol}^{-1}, \quad (2)$$

$$C_6H_{12}O_6 + 3H_2O \rightarrow CH_3COO^- + 2HCO_3^- + 2CH_3CH_2OH + 3H_2. \quad \Delta G^0 = -97 \text{ kJ mol}^{-1}, \quad (3)$$

The acetate pathway is the one with the highest theoretical hydrogen yield: 4 moles of H_2 from mole of hexose. The most efficient way according to ref. [24, 122] is the acetate pathway (1) but the most probable is the butyrate fermentation (2). Alkaline pretreatment leads most often towards acetate fermentation.

The process which could theoretically yield 12 moles of hydrogen from 1 mole of glucose:

$$C_6H_{12}O_6 + 12H_2O \rightarrow 6HCO_3^- + 6H_2 + 6H^+, \quad \Delta G^0 = 241 \text{ kJ mol}^{-1}, \quad (4)$$

is thermodynamically impossible due to positive value of Gibbs free energy.

In the case of pentoses, the reaction proceeds according to scheme (5) [27]

$$C_5H_{10}O_5 + 2.67H_2O \rightarrow 1.67CH_3COOH + 1.67CO_2 + 3.33H_2 \quad \Delta G^0 = -197.66 \text{ kJ mol}^{-1} \quad (5)$$

According to Gomez et al. [79] low loading rate and mixed reaction schemes are leading to stable dark fermentation.

4.2. Optimal temperature

The optimum temperature for the process depends on the feed type and bacterial innoculum. In the case of hydrogen generation from crop residues hydrogen yields under thermophilic conditions (T =70^{0}C) are higher than under mesophilic conditions (T= 37°C) [6, 76]. On the other hand, Azbar et al. [53] compared hydrogen generation from cheese whey: under thermophilic conditions conversion of whey to hydrogen at T= 55^{0}C was lower than in the case of mesophilic at 35^{0}C.

Zhang and Shen [147] studied mixed culture bacteria with Clostridium pasteuranium as methanogenic bacteria and hydrogen generation was stopped when the temperature reached 45°C. Mixing mesophilic culture with other bacteria does not shift optimum temperature;

35°C was still the optimum temperature for hydrogen production [147]. In the case of mesophilic Enterobacter aerogenes strain HO-39 in batch process optimum temperature was 37°C while in a continuous process 35°C [42]. According to Hawkes et al. [36] optimal temperature for a butyrate type hydrogen-production was 30°C.

4.3 Partial pressure

According to Hallenbeck [148] there is no unique answer as to whether the partial pressure of hydrogen should be close to 0 or be increased. Kramer and Bagley [81] considered that increase of hydrogen yield of more than 2 mol H_2 mol^{-1} glucose was achieved by lowering partial pressure. Kim et al. [69] obtained a yield of 1.68 mol H_2 $mole^{-1}$ glucose by lowering partial pressure. Mandal et al. [47] by lowering partial pressure from 760 mm Hg to 380 mm Hg increased yield from 1.9 mol H_2 mol^{-1} glucose up to 3.9 mol H_2 mol^{-1} glucose.

Lowering of partial pressure is obtained usually by gas sparging. Contrary to [40, 79] Mizuno et al. [113] for mixed culture of clostridium increased hydrogen yield from 0.85 to 1.43 mol H_2 mol^{-1} glucose by applying nitrogen sparging in a continuous process. In the case of argon sparging in a batch type process with Enterobacter aerogenes, hydrogen yield increased from 0.52 to 1.52 mol H_2/mol glucose [34, 50, 62]. Results of Mizuno et al. [113] are similar to results for continuous process obtained by Hussy et al. [150]. According to Hussy et al. [150] lowered partial pressure of hydrogen by the nitrogen sparging in continuous reactor, reduced the hydrogen concentration in the outflow from 50% to 7%. The decrease of off-gas concentration resulted in stable hydrogen yield of 1.9 mol H_2 mol^{-1} hexose in a 18 days period.

4.4. C/N ratio

Low C/N ratio (carbon to nitrogen ratio) is considered to inhibit dark fermentation [93]. The optimum value C/N ratio depends on the raw material, type of process and bacteria. The optimal C/N ratio should be high; for cheese whey is in the range of 30-40:1. Anzola-Rojas et al. [127] determined in the case of the wastewater optimum C/N ratio to be 137:1 and obtained hydrogen yield 3.5 mol H_2/mol sucrose. Argun et al. [89] determined for wheat powder fermentation optimal C/N ratio ~200:1.

4.5. Metal, phosphate ions

According to Wang and Wan [151] one of the most important for the process efficiency is Fe^{2+} ions. Iron ions are part of the Fe-hydrogenase enzyme that activates hydrogen generation in the anaerobic bacteria. The optimum concentration of iron ions is still not known [151]. Iron ions optimum concentration decreases with rise in temperature. Nath et al. [84] and Wang and Wan [28, 32] used $FeSO_4 \cdot 7H_2O$ solution as a source of iron ions while others preferred $FeCl_2$ [83].

According to Zhang et al. [147] the optimum iron concentration depends on the bacteria type and temperature. In the case of a mixed culture of Clostridium pasteuranium and starch as substrate, the optimum iron concentration was 800 mg l^{-1} at temperature T=25°C and resulted in a hydrogen yield of 356 ml H_2/l starch; for T= 35°C the optimum iron concentration was 200 mg l^{-1} (hydrogen yield 377 ml H_2 l^{-1}) and for T= 40°C the optimum iron concentration was 25 mg l^{-1} (hydrogen yield 351.1 ml H_2 l^{-1}). The concentration of iron ions influenced duration of fermentation: at iron ions concentration between 25-100 mg l^{-1} fermentation lasted

60 h, in range of 100 to 1600 mg l^{-1} the time decreased to 48 h [65]. Moreover, Zhang et al. [147] show that an iron concentration above 100 mg l^{-1} for Clostridium pasteuranium improved hydrogen yield from starch but it shortened the hydrogenase activity. In the case of cheese whey iron concentration in the range of 50-150 mg l^{-1} improves the generation of hydrogen [152]. According to Nath et al. [84], in the case of Enterobacter cloacae at temperature 37°C the optimum iron ion concentration was 20 mg l^{-1} (hydrogen yield was 3.31 mol H_2 mol^{-1} glucose).

Wang and Wan[52] considered that for Ni^{2+} ion optimum concentration is 0.1 mg l^{-1}, which led to a yield of 232 mg H_2 g^{-1} glucose. As the source of Ni^{2+} ion $NiCl_2$ was proposed [29].

Azbar et al. [152] investigated the influence of various metal-ions concentrations on dark fermentation yield from cheese whey. Optimal metal salt concentrations were proposed: $ZnCl_2$ (1.25–2.5 mg/l); $CaCl_2$ (250–500 m/l); $MgCl_2$ (50–100 mg l); $MnCl_2$ (2.5–5 mg/l) and $FeCl_2$ (50–100 mg/l) leading to hydrogen yield 3.5 mol H_2 mol^{-1} lactose [152].

According to Kothari et al. [99], Fang et al. [153] fragility of the bacteria increases in order with copper, zinc and nickel, cadmium , chromium and lead, respectively. However, according to Wang and Wan [151] after Shei and Lin [154] considered for sludge wastes in order zinc, copper and chromium. Phosphate ions are both good nutrition and buffer components [99]. According to Argun et al [89] optimum ratio of C/P is 2000:1.

4.6. pH and Oxidation-Reduction Potential (ORP)

The value of optimum pH depends upon the substrate and bacterial culture. The influence of pH control and dilution rate in the range 0.4 to 1.0 h^{-1} on glucose and xylose fermentation using Clostridium sp was investigated by Taguchi et al. [37]. The hydrogen production rate of fermentation increased with dilution rate under controlled pH = 6.0 conditions. The highest hydrogen production rate registered was 21.03 mmol H_2 $h^{-1}l^{-1}$ xylose for a dilution rate of 0.96 h^{-1}. Without Ph control, the maximal hydrogen production rate was 16 mmol H_2/h l xylose when the dilution rate was 1.03 h^{-1} [37]. The pH value influences the yield more than does it dilution rate, so for any dilution rate choice of optimal pH value of solution is essential.

The highest yield with pH control was 2.06 mol H_2 mol^{-1}xylose at dilution rate 0.21 h^{-1} and 2.15 mol H_2 mol^{-1}glucose at dilution rate 0.19 h^{-1} [37]. However, the role of pH control is not clear always as in the case of uncontrolled (freely evolving) pH the highest achieved yield was smaller in relation to pH controlled conditions (1.82 mol H_2 mol^{-1}xylose) at dilution rate 0.22 h^{-1} and larger (2.36 mol H_2 mol^{-1}glucose) at dilution rate 0.18 h^{-1} [37]. Xu et al. [155] recommended an acetate buffer of 110mM to 250mM as an efficient method for adjustment of pH level.

In order to control pH during dark fermentation with Clostridium sp, Zhu et al. [58] used solutions of 1.0 M of NaOH and 1.0 M HCl. Generally, optimum pH for hydrogen production by mesophilic bacteria lies in the range 5.5-6.5 while thermophilic bacteria like Clostridium thermopalmarium or Thermatoga neapolitana prefer pH in the range of 6.9-7.2 [22].

Kargi et al.[93] analyzed time evolution of pH and oxidation-reduction potential (ORP) for initial total lactose concentration 20 g l^{-1} - see Fig. 6. The data provide information on optimal pH value i.e. 5.5-6.0, for cheese whey powder fermentation under thermophilic condition. The

pH changes influence ORP value, which vary in the range from 53mV to -200 mV. According to Kargi et al. [93] the optimum ORP value is in the range -50 mV to -200 mV.

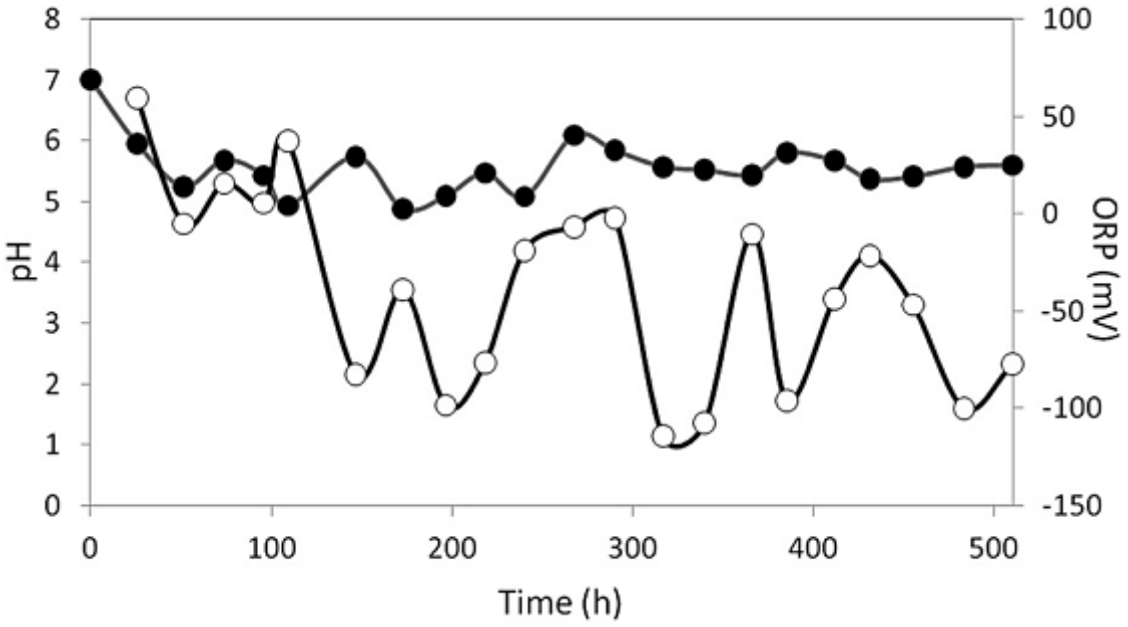


Fig. 6. Time evolution of pH (black dots) and ORP (open circles) values for substrate with initial total sugar from whey (mainly lactose) concentration 20 g l^{-1}[93]

Zhang and Shen [65] obtained an optimum pH range of 7.0-8.0 for dark fermentation of starch by Clostridium pasteuranium. Zhu et al. [58] determined optimum pH=5.0 for fermentation of pig manure by Clostridium sp. According to Yokoi et al. [42] optimum pH is in the range 6.0-7.0 for fermentation caused by Enterobacter aerogenes strain HO-39.

The value of pH can influence the mechanism of bacteria growth. At pH between 3.3-4.0. Enterobacter bacteria grow aerobically while above this range anaerobically [156]. Hussy et al. [68] claims that the pH in the range from 4.5 to 5.2 is undesirable for fermentation of sewage sludge from wheat flour industry. In this range of pH hydrogen can be consumed by homoacetogenesis and propionate producing processes [68]. Optimum pH for Enterobacter cloacae DM11 is 6.5 [84]. According to Vijayaraghavan and Ahmad [157] optimal hydrogen yield was 4708 ml H_2 l^{-1} for palm oil mill waste fermentation achieved at pH 5.0. According to Ren et al. [45] pH below 4.5 leads during hydrogen fermentation to ethanol production.

4.7 Hydraulic retention time (HRT)

According to Zhu et al. [72] hydraulic retention time plays a significant role in the case of the semi-continuous fermenter. A change of HRT can cause a variation of hydrogen concentration in the gas outflow - Fig. 7.A. The dependence of biogas production on pH and HRT is shown in Fig. 7.B.

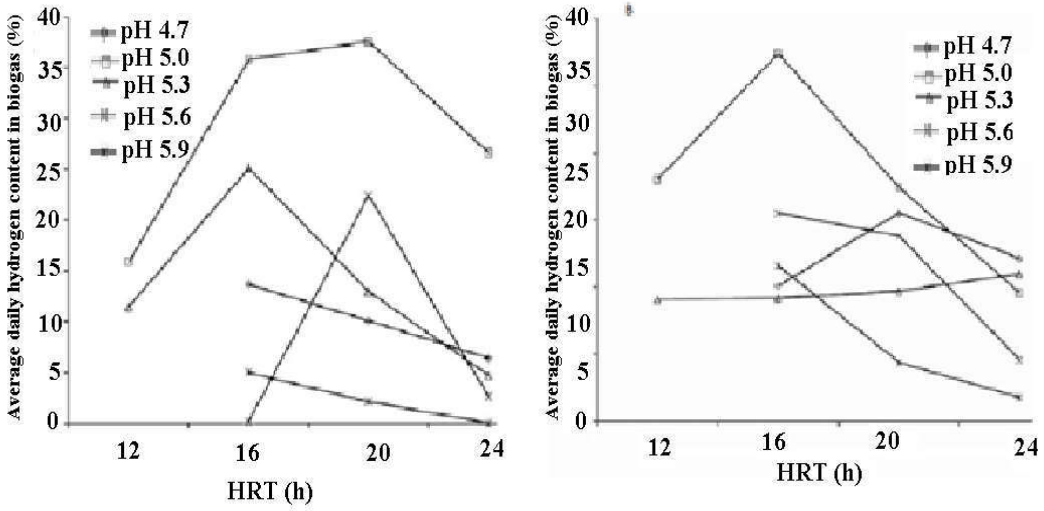


Fig. 7. Influence of change HRT, and pH to hydrogen content (A), biogas production B after Zhu et al. [58].

As it is shown in Fig. 7 A the highest concentration of hydrogen in biogas was observed for HRT in the range 16-20 h at 35.7-37%[58]. Maximal gas production of 27 l/day was registered at pH 5. Optimal hydraulic retention time for hybrid reactor series of Wu and Chang [74] was in the range 4-8 [h].

According to Lee et al. [134] reduction of hydraulic reduction time increases hydrogen production rate from sucrose independently from reactor's height to diameter ratio. Chen et al. [111] decreased hydrogen production rate from sucrose from 0.094 mol $H_2 \cdot h^{-1}$ to 0.032 mol $H_2 \cdot h^{-1}$ by increasing HRT from 6 to 13.3 h.

According to Xing et al. [158] low HRT is desired for hydrogen production especially in CSTR. Low HRT enables the removal of methanogenic bacteria from sludge due to its short specific growth time. The HRT value correlates with dilution rate. According to Chen et al. [111] dilution rate should be in the range of 0.075 h^{-1} to 0.167 h^{-1} for efficient hydrogen production in CSTR. Chen et al [110] decreased HRT from 13 h to 3.3 h improving hydrogen gas production from 4.9 to 26.9 l H_2 l^{-1} of sucrose. However, a decrease of HRT to values lower than 3.3 h lowered the hydrogen production [110].

Wu et al. [159] studying continuous anaerobic process determined HRT to be 0.5 h for the semi-continuous reactor with sewage sludge. However, the optimum HRT value was 6 h when pH = 5.7. Oh et al [55] and Logan et al. [101] have reduced HRT from 5h to 3.3h in a membrane reactor; this led to increased conversion of glucose from 90% to 98%. The decrease of HRT can lead to lower pH [158]. The dependence results from an increased accumulation of volatile fatty acids. However, pH can be adjusted by 0.1 M NaOH solution like in Chen and Lin[110]. In the case of the membrane reactor, the retention time can be divided into sludge retention time and hydraulic retention time. The increase of sludge retention time from 3.3 h to 12 h improved efficiency of glucose to hydrogen conversion from 22 to 25% [160].

In the case of UASB reactor decrease of HRT from 24 to 2 h lead to lower hydrogen yield from 2.14 mol H_2 mol^{-1}glucose to 1.83 mol H_2 mol^{-1}glucose. However, the reduction of HRT increased glucose conversion to 85% [160].

In the fluidized bed reactor using sucrose as substrate optimal HRT was 2 h with hydrogen yield 1.34 mol H_2 mol^{-1} hexose [161].

In the case of packed-bed reactors, optimal HRT depends on carrier and immobilized bacteria cultures. The HRT value changes from 0.5 h when sludge is packed with activated carbon to HRT =10 h when the carrier is from glass/spongy beads [116]. For a packed-bed with Enterobacter aerogenes HU 101, optimal HRT was 0.67 h, while for the mutant bacteria AY-2 it was 0.55 h [162]; at the same time the hydrogen production from sucrose changed from 31 to 58 mmol H_2 $l^{-1}h^{-1}$ for the mutant.

For fixed bed reactor optimal HRT also depends on the material of matrices that immobilized the bed. In the case of support from activated carbon optimal HRT was 1 h while for expanded clay it was 2 h [126].

5. Separation methods of hydrogen from the dark fermentation products

The most common conventional method for hydrogen separation is pressure swing adsorption (PSA). It depends on an adsorbent bed that captures the impurities in the waste gas stream under high pressure while the impurities release at low pressure. Multiple beds are used simultaneously, in order that a continuous separation of hydrogen can accrue up to a purity of 99.9%. Another method is temperature swing adsorption (TSA), which is different from PSA and based on adsorption under high temperature. This method is not widely used due to the need of a relatively long process of heating and cooling of sorbents. Another new process is electrical swing adsorption based on the use of electric field to drive hydrogen separation from the gases mixture. In this process, the switching between adsorption and desorption works like on/off switching, which reduces the need to transport or heat sorbent materials. The cryogenic process was also used to purify hydrogen, but it needs very low temperatures, therefore, they are relatively expensive [163].

5.1. Hydrogen separation using membranes.

The mechanism of membrane separation is based on selective penetration of hydrogen through the membrane, with selectivity depending on the membrane properties. The partial pressure of hydrogen in the feed stream is the driving force for permeation, which is balanced by the partial pressure of hydrogen in the permeate stream.

The selectivity of hydrogen penetration through the membrane depends on the membrane material. There are two types of membrane: organic (polymer or carbon) and inorganic (metallic or ceramic). The purity of hydrogen reached 99.99% through dense metallic membranes, especially through Pd and its alloys, but there are limitations for metallic membranes due to: (i) poisoning effect of hydrogen sulfide (H_2S) and other feedstocks have on the hydrogen transport mechanism, (ii) high cost for the preparation of Pd membranes and (iii) mechanical stability [164]. The key advantages of polymer membranes are their ability to withstand high pressure drops and their low cost. Therefore, the separation of H_2 by polymeric membranes has become an attractive technology.

The transport of hydrogen through dense membranes occurs via the diffusion mechanism, comprised of three main steps:

1. sorption of the gaseous penetrants at the upstream side of the membrane,

2. diffusion of the penetrants across the membrane,
3. desorption of the penetrants at the downstream side of the membrane [165,166].

The mechanism is driven by a difference in the thermodynamic activities existing at the upstream and downstream faces of the membrane as well as the interaction between the molecules that constitute the membrane material and permeating molecules.

5.2. Selection of polymeric membrane materials

A membrane separation of hydrogen from various mixtures of gases (including those generated during dark fermentation process) can provide the best performance depending on the membrane polymeric material. The polymers which are used for membrane preparation may be both glassy and rubbery polymers [167]. Usually, when rubbery polymers are used for membrane preparation high permeability with a relatively low selectivity results. When glassy polymers are used for membrane preparation, this leads to high selectivity and lower permeability with high product purity of membranes [168]. Examples of polymers that can be used to prepare membranes for gas separation are shown in Table 4.

Table 4. Polymeric materials and their characteristics

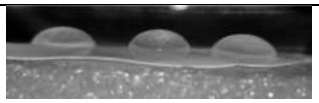
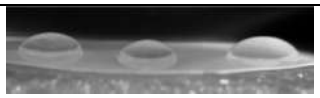
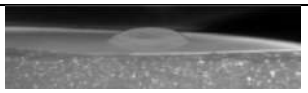
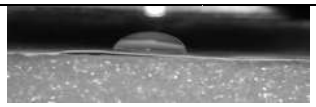
Polymers Materials	***Glass transition temperaure (Tg, K)***	***Density g/cm^3***	***Ideal Selectivity of H_2/N_2***	***Ideal selectivity Of H_2/CO_2***	***References***
Cellulose acetate (CA)	243	1.3	12.52	0.4	[169]
Poly(vinylidene fluoride) (PVDF)	238	1.75	3.42	2	[170]
Polydimethylsiloxane (PDMS)	150	0.97	2.2	0.2	[170]
Polysulfone (PSU)	459	1.24	56	2.5	[166]
Poly(ether sulfone) (PESU)	498	1.37	69.5	2.7	[171]
Poly(phenylene oxide) (PPO)	483	1.06	29.7	1.5	[170]
Polyimide (PI); Matrimid	502	1.24	97	3.9	[172]

5.3. Implementation of nano-materials in gas separation-membranes

The asymmetric PES/Mn(acac)3 blend membranes were successfully fabricated by the phase inversion method and by application of the metalorganic compound Mn(acac)3 in the polymer solution mixture. The addition of Mn(acac)3 resulted in a reduction in pore size, porosity and low contact angle due to an improvement in hydrophilicity in relation to bare PES. The tensile strength of the prepared membrane was 57.8 kg/cm^2 with an elongation of 6.2% [173].

Nanoparticle materials such as titanium oxide were used to produce a PES/TiO_2NTs blend membrane. This membrane was used to separate water vapour, where the selectivity of water vapour reached up to 100% and the average flux of 18.2 kg/m^2 h. A small addition of titanium oxide nanotubes decreased the contact angle as shown in Table 5 [174].

Table 5. Membrane porosity and contact angle of bare PES and PES/TiO2NTs blend membranes

TiO_2NTs %	*Porosity (%)*	*Contact angle*	*Membrane wettability photos*
0.18%	*61*	*75°*	
0.35%	*73*	*65°*	
0.53%	*79.4*	*55°*	
0.85%	*91*	*45°*	
bare PES	*27.7*	*85°*	

5.4. Hydrogen separation system using membrane technique

The design of the feed part of the apparatus enables pure and mixed gas permeation experiments, at predetermined gas concentrations and flow rates [175] - see Fig. 8. The required concentration of gases in the mixture is obtained using mass flow controllers. The gas mixture passes through the polymeric membrane film sandwiched in the membrane test module, which has three openings: for feed, retentate and permeate flux. The last leaves the system via the backpressure regulator to control the total pressure on the feed side of the membrane. The membrane system is located in a water bath to facilitate studies of the effect of temperature and to indicate possible gas leakage. The gases are tested using an IR or GC analyzer.

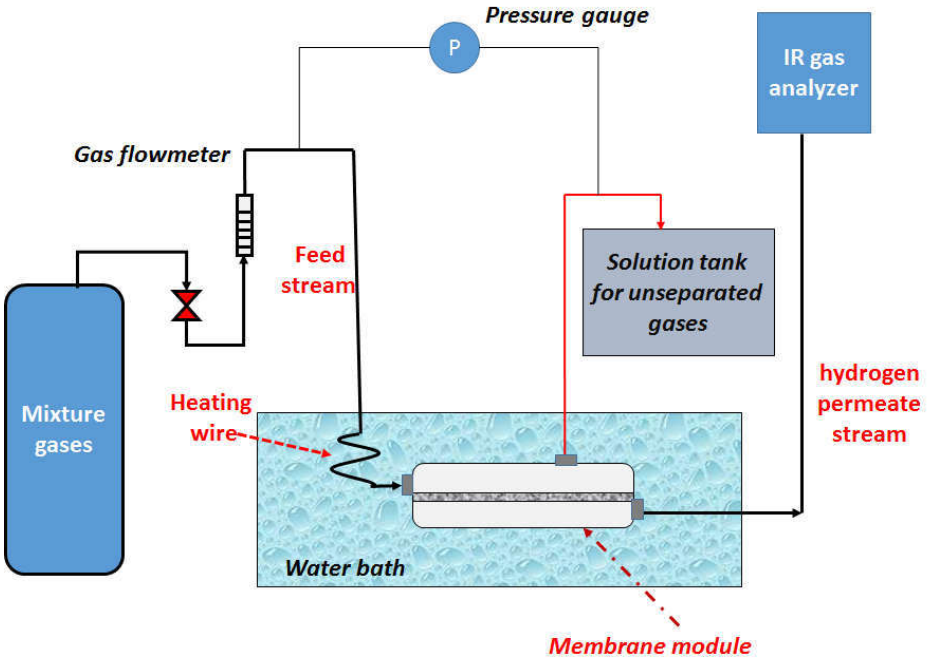


Fig. 8. System for hydrogen separation by membrane

5.5 Membrane Modules - Efficiency and Cost Saving

A membrane separation is one of the commercially available separation methods that can be used to minimize wastes, equipment requirements and to improve product quality. The membrane systems are particularly effective for recovery and reuse of liquids, gases and solids. The use of a membrane system can lead to reduction of cost and industry environmental-impact as well as to increased competitiveness [176].

Membranes can be found in four main configurations: tubular, spiral wound, plate-and-frame, and hollow fibre. The simplest module is the plate-and-frame setup which resembles conventional filtration; it consists of a flat sheet membrane in addition to spacers between the membranes to prevent dynamic adhesion between them. The tubular membrane configuration consists of a multi-tubes set, where the feed is pushed around tubes, while the product is permeating inside the tube. The spiral wound element is the most widely used configuration in the market for commercial applications such as nanofiltration and reverse osmosis application. This module is basically a flat sheet membrane but it is wrapped around a particular tube [176]. The feed flows through the membrane and the permeate is collected on the other side of the membrane then twists or spirals into the centre of the tube. The hollow-fibre membrane module composed of a bundle of hollow fibres is placed in a certain container; the feed is pushed under pressure to pass through the fibre and the permeate is collected at the end of the bundle.

The characteristics of modules for gas separation depend largely on membrane material and structure [176]. An industrially useful membrane module must exhibit several properties such as high separation efficiency, high permeation flux, good mechanical stability, tolerance to temperature variation, fouling resistance and low costs. The operating units must contain a number of modules to provide the required membrane surface area for effective separation. Each module must have an inlet and an outlet plus a permeate port and a cleaning system if appropriate. Modules may be arranged in either series or parallel units depending on the applications [176,177]. Table (6) illustrates the typical characteristics of membrane modules.

For example, the membrane CO_2-separation-technique has lately attracted considerable attention owing to the new regulations on carbon dioxide emissions. These resulted in rapid development of CO_2 capture technologies to be used in existing and new power plants with the goal of achieving 90% CO_2 capture. The aim was for a limited increase of electricity costs - no more than 35% [178]. The power and hydrogen production units as well as heating systems, especially in the steel and cement industries, etc. are examples where carbon dioxide is produced in huge amounts. The use of membrane modules for gas separation in these industries can reduce the cost of the procedure [179].

Table 6: Typical characteristics of membrane modules

Membrane module	Packing density m^2/m^3	Common application	Relative investment cost
Hollow fibre	600-1200	ultrafiltration, gas separation	medium
Spiral-wound	300-1000	ultrafiltration, nanofiltration, reverse osmosis, pervaporation, gas separation	low
Tubular	<100	filtration of high solids content streams	very high
Plate-and-frame	100-600	reverse osmosis, pervaporation, gas separation	high

5.6. Membrane fouling

In spite of the merits of membrane-based separation process, fouling is a major and serious problem limiting the use of membranes in a wide range of applications [176]. Fouling refers to irreversible precipitation of organic or inorganic materials, suspended particles or bacteria on the surface of the membrane and in its pores, which results in a decline of the permeate flux and an increase in hydraulic resistances [176]. There are four different types of fouling: organic, scaling, biofouling and colloidal fouling. Organic fouling results from organic compounds such as hydrocarbons, humic or fulvic acids, etc., which coat the surface of the membrane or clogs the pores of the top membrane layer [176]. Scaling fouling comes from a precipitation of salts or inorganic compounds on the membrane surface. When the concentration of an inorganic compound exceeds its solubility limit it is deposited on the surface of the membrane. The scaling results in a higher pressure drop across the filtration system, with a decline of separation efficiency (and permeate flux) leading to low quality of the product. Calcium carbonate ($CaCO_3$) is an example of a common scale formed on a RO membrane. Microbial contamination of feed leads to bacterial adhesion on the membrane surface and creation of biofilm called biofouling which increases the hydrolytic resistance to penetration of permeate through a membrane. Colloidal fouling results from insoluble particles such as silica or clay, gathering on the membrane surface. The fouling limitation and control can be achieved by some pre-treatment procedure, using anti-scaling materials or an addition of disinfectants [176].

For dense membranes which are used in gas separation, two types of fouling can be identified: surface blocking and particle blocking. In the case of surface blocking, some of the membrane

area is not able to transport any mass and, therefore, the active membrane area is reduced. That can also lead to surface blinding. The flux through the partly blocked membrane can be described as a function of the theoretical flux through the unblocked membrane by:

$$J(t) = J_0 * \frac{(A_t - A_{bl*}(t))}{A_t} \quad (6)$$

where J_0 is the flux through the unblocked membrane, A_t is the total membrane area, A_{bl} is a blocked area of membrane and t is time. In the case of particle blocking there are two contributory effects: a reduction of active membrane-area and an increase of the diffusion path through the membrane [180].

During gas separation the membrane fouling is not caused only by the wetting but also by synergetic effects of water vapour with gaseous components: SOx, NOx or fine particles in the flue gas. For example, a membrane performance suffers a notable reduction after exposure to SO_3 and particles in a wet gas stream. The effects of SO_3 and particles is irreversible, whereas the fouling by SO_2 and NO_2 can be reversed by N_2 sweeping. The investigations of membrane modules, used for gas separation, confirm that they are polluted by gaseous and particle impurities to various extents. However, during the CO_2 separation from flue gas containing fine particles, the pores of the membrane are blocked and the surface is covered by a particle layer. These fine particles occupy a significant area and lead to increased mass-transfer resistance and reduced membrane performance [177,181].

To overcome these problems, a number of methods have been developed by designing special modules, several pretreatment processes and membrane modification. The most effective and relatively low-cost method is membrane surface treatment or modification. It is widely known that hydrophilicity, charge and roughness of the membrane surface greatly influence membrane fouling. So, much effort has been expended by many researchers to modify and enhance surface hydrophilicity of membranes. These methods included blending with hydrophilic polymers, decrease of physical adsorption, surface coating, grafting and plasma polymerization, which resulted in extended membrane lifetime [176]. The membrane modification techniques include [176–181]:

- the bulk modification of the membrane matrix, such as blending and copolymerization and
- the modification of the membrane surface, such as grafting of certain hydrophilic monomers at the surface or chemical treatment to introduce polar groups on the membrane surface or coating with hydrophilic materials.

6. Summary

Dark fermentation is a process of microbial anaerobic conversion of simple organic carbohydrates or glycerol molecules into short-chain organic acids, carbon dioxide and, most importantly, hydrogen molecules. The standard dark fermentation as a direct source of hydrogen processes glucose, while extended dark fermentation can make use of any simple organic compounds or mixture of organic compounds.

The bacteria used to produce hydrogen need to possess special enzymes - hydrogenases. The most efficient hydrogen production occurs with strict bacteria, including clostridium. Other bacteria, called facultative, produce hydrogen less efficiently but they are more oxygen resistant. Facultative bacteria include: Enterobacters, Baccilus and Citrobacters.

Particularly if they are stressed, the bacteria activate hydrogenase, in defence as an emergency action. Bacteria need to retain carbon that will be used to rebuild bacterial structure. It can be illustrated in the case of clostridium perfringens, at so called gas gangrene or clostridial myonecrosis stage. Bacteria produce hydrogen as metabolite because they are on alert and retain carbon for possible rebuilding after contact with victims' defense systems like antibodies (leucocytes - a stress agent for bacteria). Therefore, for efficient hydrogen production bacteria need to be pretreated using one or more of the following stress factors:

physical: ultrasonication, microwave, shaking (centrifuging), heat (heat-shock or thawing), or

chemical: pH change (acid/base pretreatment; for example linoleic acid, chloroform).

The yield of hydrogen production varies in different reactors. These are special vessels designed to provide optimal conditions for bacteria multiplication and substrate distribution in order to enable efficient hydrogen production. Batch type reactors are the primary option due to their simplicity, high conversion–level of substrate and small pretreatment requirements. A disadvantage of batch type reactors is their generally low production rate. The semi-continuous process can be used for preparation of bacterial cultures for continuous process that prevents wash-out in a continuous stage. Advantages of CSTR reactors are: high mass transfer due to mixing, simplicity of construction and operation. The CSTR process is limited by low biomass concentration, cell retention in low dilution rate and risk of washout of cells at a high dilution rate. Membrane reactors increase conversion by separation of products from substrates. Fluidized bed reactors allow for use of high volume fraction of biomass without the risk of attrition which occurs with CSTR. Besides, bubble column like reactor allows for high mass transfer. Packed-bed keeps biomass in the reactor, preventing its washout like in CSTR. In the fixed-bed reactor a biofilm typically replaces the catalytic layer (fixed bed) in reactors. The advantages are generally low pressure drop, and the simple and robust construction. Another advantage is the high magnitude of volumetric mass transfer coefficient. The disadvantage of the fixed-bed reactor is its limited surface area. UASB reactors are characterized by conversion of biomass in large quantity and using high organic load. The main disadvantage of the UASB reactor is its sensitivity to the channeling effect causing loss of contact with the substrate and the bed. The CIGSB reactor allows improvement of the mixing properties by applying different variations of agitation systems (physical, mechanical) and different height to diameter ratio of the reactor. In the case of a multi-stage process, the feed preparation and hydrolysis are kept under different conditions from the main process.

The relevant parameters for the dark fermentation process are: feed, temperature, partial pressure, C/N ratio, pH and ORP, metal ion concentration and HRT. The most important metal ion seems to be the iron ion because it is the necessary element of most hydrogenase enzymes. Other metal ions mentioned in the literature are Ni^{2+}, Mg^{2+}, Ca^{2+}, Mn^{2+}, Zn^{2+}, and Co^{2+}. Optimum values of parameters depend on the bacteria genus and substrate type.
Different polymeric membranes were discussed and a membrane hydrogen separation system was designed. Implementation of nanoparticles to the membrane preparation process is described. It was found that a small addition of titanium oxide nanotubes decreased the contact angle.

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