



Review

Anaerobic biofilm reactors for dark fermentative hydrogen production from wastewater: A review



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HIGHLIGHTS

- A review on anaerobic biofilm reactors for bio-H₂ production from wastewater.
- Effect of substrate type, substrate concentration, and hydraulic retention time.
- Effect of carrier material, inoculum type and pretreatment, temperature, and pH.
- Investigation of microbial dynamics, fermentation pathways, and metabolites.

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ABSTRACT

Dark fermentation is a bioprocess driven by anaerobic bacteria that can produce hydrogen (H₂) from organic waste and wastewater. This review analyses a relevant number of recent studies that have investigated dark fermentative H₂ production from wastewater using two different types of anaerobic biofilm reactors: anaerobic packed bed reactor (APBR) and anaerobic fluidized bed reactor (AFBR). The effect of various parameters, including temperature, pH, carrier material, inoculum pretreatment, hydraulic retention time, substrate type and concentration, on reactor performances was investigated by a critical discussion of the results published in the literature. Also, this review presents an in-depth study on the influence of the main operating parameters on the metabolic pathways. The aim of this review is to provide to researchers and practitioners in the field of H₂ production key elements for the best operation of the reactors. Finally, some perspectives and technical challenges to improve H₂ production were proposed.

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1. Introduction

The current global economy is strongly dependent on fossil fuels and all perspective studies show that they intend to exhaustion. Moreover, their use to produce energy poses several environmental problems such as pollution and production of greenhouse gases. Therefore, research on alternative renewable clean-energy resources has become a priority for politics and scientists in the last decades (Dunn, 2002). Nowadays, hydrogen gas (H₂) represents a promising alternative to fossil fuels, as this offers the possibility of generating a valuable energy carrier that is renewable and carbon neutral. Various processes are commonly employed to produce H₂, including electrolysis of water, photolysis, photo fermentation and dark fermentation. Compared

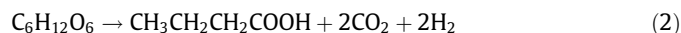
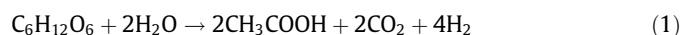
to other processes, bacterial dark fermentation appears to be one of the most attractive processes (Nath and Das, 2004), because it employs the ability of strict or facultative anaerobes such as *Clostridium* or *Enterobacter* to produce H₂ and volatile fatty acids (acetic acid, butyric acid, propionic acid, etc.) from complex organic feedstocks. This indicates a very interesting potential market of organic waste valorisation for the production of volatile fatty acids and H₂ (Singhania et al., 2013). As reported in the literature, carbohydrates are the most effective substrate for dark fermentative H₂ production (Show et al., 2012). Therefore, carbohydrate-rich waste and wastewater, such as food manufacturing wastes, cheese whey, sugar factory wastewater, rice winery wastewater, etc., appear to be suitable feedstocks for the production of H₂ (Lin et al., 2012).

Several international studies have investigated fermentative H₂ production via batch experiments using synthetic solutions made of simple carbohydrates such as glucose and sucrose (Fang and Liu, 2002; Chen et al., 2006; Castro-Villalobos et al., 2012). Overall, they found that H₂ production usually followed two main metabolic

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pathways: (i) the acetate pathway (Eq. (1)), and (ii) the butyrate pathway (Eq. (2)).



Depending on the pathway, the theoretical biogas composition is around 67% of H_2 (acetate pathway) or 50% of H_2 (butyrate pathway). This indicates a very interesting potential of H_2 production from carbohydrate-rich wastewater.

The technical feasibility of H_2 production from various types of synthetic and real wastewaters has been largely tested in continuous and semi continuous reactors (Li and Fang, 2007; Lin et al., 2012). In fact, continuous reactors are usually preferred than batch reactors for full scale application because, if well designed and operated, they may give continuous biogas production under steady-state conditions, thus avoiding the feeding and emptying steps of batch reactors (Show et al., 2011). Two main typologies of reactors were commonly employed for continuous and semi continuous experiments (Jung et al., 2011): (i) suspended biomass reactors, in which the fermentative bacteria form suspended flocks of biomass that are continuously mixed with wastewater, and (ii) biofilm reactors, where the fermentative bacteria grow on the surface of the carrier material, forming a biofilm. Among the suspended biomass systems, the most used for H_2 production are the anaerobic sequencing batch reactor (ASBR) and the continuous stirred tank reactor (CSTR), whereas among the biofilm systems the most used are the anaerobic packed bed reactor (APBR) and the anaerobic fluidized bed reactor (AFBR). Suspended biomass systems present the advantage to treat mixtures of wastewater and solid wastes. In addition, the complete mixing improves mass transfer and the contact between the biomass and substrate. However, biomass concentration in ASBRs and CSTRs is limited, because the suspended biomass has the same retention time as the hydraulic retention time, and it is flushed out with the effluent (Li and Fang, 2007). Biofilm reactors can overcome the drawbacks of suspended biomass reactors by decoupling biomass retention time from hydraulic retention time, thus increasing biomass concentration in the reactor (Jung et al., 2011). This work presents a critical review of a relevant number of recent papers that have investigated H_2 production in APBRs and AFBRs. The effect of several parameters, including the substrate type and concentration, hydraulic retention time, pH, temperature, inoculum type and pretreatments, on H_2 production performances was evaluated by an in-depth critical analysis of the main results from the reviewed papers. This review provides to researchers and practitioners in the field of dark fermentative H_2 production key elements for the best operation of the reactors.

2. Reactor design and process parameters

2.1. Type of reactor and total volume

The main design parameters and experimental results from relevant studies that have investigated H_2 production in APBRs and AFBRs are summarised in Tables 1 and 2, respectively. The major difference between APBR and AFBR is related to the hydraulic behaviour of the bed. Carrier material for biofilm growth in APBR is fixed, and the water passes through the void volumes of the bed, according to two main possible flow directions: vertical (up-flow or down-flow) or horizontal. Instead, the flow direction in AFBR is always vertical up-flow, and the flow velocity is equal or greater than the minimum velocity for which the upward drag force exerted by the fluid is equal to the apparent weight of the particles in the bed, thus leading to the suspension of the carrier

material ("bed fluidisation"). Bed fluidisation favours the contact between biofilm and substrate, thus improving mass transfer and treatment efficiencies. Instead, the hydraulic mixing regime in APBRs is usually less turbulent than in AFBRs, thus resulting in a higher mass transfer resistance (Show et al., 2011). As shown in Tables 1 and 2, the total volume of the reactors ranged from 0.075 to 34 L. However, most of the studies were performed with reactors of volume between 0.5 and 5 L, and only one known study was conducted with a total reactor volume over 10 L (Babu Pasupuleti et al., 2014). The working volume is an important parameter when evaluating the water treatment capacity of a reactor, as it indicates the volume of water that the reactor may contain per unit of void hydraulic retention time (HRTv). In the reviewed studies, the working volume of the reactors was usually 50–70% lower than the total volume, thus depending on several parameters such as height and porosity of the bed, and on the volume of the void zone that is dedicated to the collection and separation of the biogas at the head of the reactor. Overall, most of the reactors were conceived with a vertical-flow cylindrical geometry and a ratio of height (H) to diameter (D) higher than 2. The use of H/D ratios higher than 2 should promote the dissipation of initial turbulence and improve the hydraulic performance of packed beds.

2.2. Carrier material for biofilm growth

A large variety of materials, including glass beads, expanded clay, activated carbon, ceramic fittings and many types of synthetic polymeric materials, were tested as carrier materials for biomass growth in APBRs and AFBRs (Tables 1 and 2). Most of these materials are chemically inert, highly porous and have good surface properties such as high specific surface, roughness and low surface energy, which are decisive physico-chemical properties for the initial adhesion and growth of the biofilm (Habouzit et al., 2011). The shape of the carrier materials was usually granular, spheroidal or cylindrical. The size of the carrier materials used in APBRs ranged from 1.5 to 25 mm, and their density from less than 0.5 up to 2 g/cm³. Instead, the size of carrier materials used in AFBRs was comprised between 0.2 and 4 mm, and their density between 1.05 and 1.50 g/cm³. Overall, size and density of the carrier materials used in AFBRs were lower than those in APBRs. In fact, it is well known that the minimum flow velocity that is needed for bed fluidisation decreases with decreasing size and density of the particles (Barros and Silva, 2012), thus reducing the energy consumption that is required to fluidise the bed. Furthermore, in most of the studies, the ratio of diameter of the reactor (D) to diameter of carrier material (d) was higher than 10. Indeed, D/d ratios higher than 10 should limit wall effects and improve hydraulic performances of packed bed reactors (Zeiser et al., 2001). Intra particle porosity and specific surface area of the carrier materials varied between a large range, from 12 cm²/g for low density polyethylene pellets (Ferraz Júnior et al., 2014) to 1100–1350 m²/g for activated carbon (Zhang et al., 2007; Guo et al., 2008).

2.3. Type of inoculum and pretreatment

Seed sludge from wastewater treatment plants (WWTPs) was used as inoculum for biofilm growth in most of the studies (Tables 1 and 2). Only few studies were conducted with pure cultures (Yokoi et al., 1997; Rachman et al., 1998; Zhang et al., 2006; Fritsch et al., 2008; Peintner et al., 2010). Processes using seed sludge are usually preferred for pilot scale application, because they are easier to operate and to control compared to pure cultures (Li and Fang, 2007). However, sludge from WWTPs contains a large variety of microorganisms, including H_2 producers and H_2 consumers like methanogens (Wong et al., 2014). For this reason, the sludge was usually pretreated to suppress as much as

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