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Pre-treatment technologies for dark fermentative hydrogen production: Current advances and future directions

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ABSTRACT

Hydrogen is regarded as a clean and non-carbon fuel and it has a higher energy content compared to carbon fuels. Dark fermentative hydrogen production from organic wastes is the most promising technology for commercialization among chemical and biological methods. Using mixed microflora is favored in terms of easier process control and substrate conversion efficiencies instead of pure cultures. However, mixed cultures should be first pre-treated in order to select sporulating hydrogen producing bacteria and suppress non-spore forming hydrogen consumers. Various inoculum pre-treatments have been used to enhance hydrogen production by dark fermentation including heat shock, acid or alkaline treatment, chemical inhibition, aeration, irradiation and inhibition by long chain fatty acids. Regarding substrate pre-treatment, that is performed with the aim of enhanced substrate biodegradability, thermal pre-treatment, pH adjustment using acid or base, microwave irradiation, sonication and biological treatment are the most commonly studied technologies. This article reviews the most investigated pre-treatment technologies applied for either inoculum or substrate prior to dark fermentation, the long-term effects of varying pre-treatment methods and the subsequently feasibility of each method for commercialization.

1. Introduction

Hydrogen (H_2) has been termed "fuel for the future". It is an eco friendly energy carrier since water is the only product of its combustion and it has the highest energy content (122 kJ/g) and heating value (142 kJ/g) among all common gaseous fuels (Argun et al., 2008; Kapdan and Kargi, 2006). It may be used for electricity generation by microbial fuel cells or used directly as a fuel for vehicles (De Gioannis et al., 2013; Goud et al., 2011; Kapdan and Kargi, 2006; Premier et al., 2013). The majority of hydrogen is produced through non-biological methods including steam reforming or thermal cracking of natural gas, partial oxidation of heavy hydrocarbons, coal gasification, thermal and thermo chemical decomposition of organic matters, electrolysis and photolysis. All these processes are energy intensive and expensive. A more sustainable option would be biological H_2 production using renewable substrates. The biological H_2 production methods are including photolysis, photo fermentation and dark fermentation. Photolysis and photo fermentation are performed by cyanobacteria and algae and need sunlight and water to produce H_2 thorough photosynthesis. Photolysis would be inexpensive and sustainable only if wastewaters are used as substrate. Similar to photolysis, for photo fermentation also light is required but the substrates are organic compounds which are degraded to H_2 and CO_2 by purple bacteria (Azwar et al., 2014).

Another possible route for H_2 production is anaerobic degradation of organic substrates by heterotrophic bacteria in a process called dark fermentation (DF). DF is regarded as the preferred method among biological means for H_2 production due to higher yields and lower operational costs (Hallenbeck et al., 2012; Mathews and Wang, 2009). H_2 can be produced both by pure and mixed cultures; though, using mixed cultures is a more strong strategy for full scale applications firstly because it is more economic (no sterilization costs and easier process control) and secondly for enhanced substrate degradability due to presence of a wide variety of microorganisms (Brenner et al., 2008). However, presence of different groups of H_2 consumers (methanogens, homoacetogens and sulphur reducing bacteria), or propionic acid and lactic acid bacteria (which convert substrate to other products) are drawbacks for using mixed cultures. Hence, mixed cultures first should be pre-treated in order to select hydrogen producing bacteria and limit growth of hydrogen consuming microorganisms.

H_2 can be produced mainly from carbohydrates degradation to volatile fatty acids (VFAs) by anaerobic microorganisms in pH

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range of 4–10 (reactions 1 and 2) (Thauer et al., 1977). Theoretical H_2 yields are 4 and 2 mol mol^{-1} glucose when substrate is converted to acetic and butyric acid respectively without any microbial growth. Propionic acid and lactic acid producing pathways are not accompanied with H_2 production (reaction 3 and 4). Usually a mixture of VFAs may be produced during DF with acetate and butyrate as the main products (Alibardi and Cossu, 2016; De Gioannis et al., 2013; Ghimire et al., 2015a; Pan et al., 2008; Pendyala et al., 2012). However, when the pH is lower than 5, ethanol- type fermentation may occur in which main soluble products are ethanol and acetic acid (Ren et al., 2007).

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \quad (1)$$

$$C_6H_{12}O_6 \rightarrow CH_3(CH2)_2COOH + 2CO_2 + 2H_2 \quad (2)$$

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3CH2_2COOH + 2H_2O \quad (3)$$

$$C_6H_{12}O_6 \rightarrow 2CH_3CHOHCOOH \quad (4)$$

Using renewable substrates including organic wastes is a promising strategy in terms of reducing process costs and makes the process more sustainable (Hallenbeck, 2009; Hallenbeck et al., 2012). Moreover, the VFA-rich effluents after DF are regarded as suitable substrates for either photo fermentative H_2 production or two-stage anaerobic digestion. Total VFAs concentrations in the range of 3000–5000 mg/L has been reported in the literature at the end of dark fermentation (Cheng et al., 2011; Contreras-Dávila et al., 2017; Dong et al., 2010; Giordano et al., 2014; Veeravalli et al., 2014). Although H_2 yield with DF is higher compared to other biological methods, it is still low for commercialization. Many factors should be considered in DF in order to optimize the process. Pre-treatment technologies are known as essentials of DF to overcome obstacles responsible for low H_2 yield. Pre-treatments technologies employed for DF are classified into inoculum and substrate pre-treatments. Inoculum pre-treatment technologies are aimed at selecting H_2 producing microorganisms and therefore increased H_2 production while the goal for substrate pre-treatment is enhancement in H_2 yield through better hydrolysis of complex substrates and provide biodegradable nutrients for microbial growth and H_2 production. This paper reviews 1) the influence of the most commonly investigated inoculum pre-treatments on inhibition of H_2 consumers in dark fermentation, 2) varying substrate pre-treatment technologies employed for increased solubilisation of low degradable compounds and 3) advantages and disadvantages of each pre-treatment in terms of productivity as well as economic and technical feasibility.

2. Inoculum pre-treatment methods

Inhibiting H_2 consuming microorganisms such as hydrogenotropic methanogens, homoacetogens, lactic acid bacteria, propionate producing bacteria and sulfate reducers is one of the main steps for dark fermentative H_2 production when using mixed microbial communities (Saady, 2013). Presence and growth of varying H_2 consumers depends on many factors and, therefore, may vary between different culture conditions. For instance, lactic acid and propionic acid bacteria dominate in conditions such as high loading rates (Oh et al., 2004). Nevertheless, hydrogenotropic methanogens have the biggest contribution for H_2 consumption among all H_2 consumers and their presence in mixed microflora reduces the H_2 yield significantly. Without inhibiting methanogens, H_2 will be consumed by them to produce methane. Considering this issue, the main goal of inoculum pre-treatment is enriching H_2 producing bacteria and suppresses H_2 consuming ones and mainly methanogens. The principle of inoculum pre-treatment technologies is that H_2 producers (mainly *Clostridium* spp. and *Bacillus* spp.) can sporulate when they are subjected to harsh environmental conditions of pH, temperature, irradiation, chemicals, etc. (Gunsalus et al., 1978; Ren et al., 2008; Wong et al., 2014; Zhu and Béland, 2006). Therefore, they can survive in such extreme conditions whilst non-spore-forming H_2 consumers that are not resistant to sever environmental conditions will be destroyed. Sporulating bacteria are able to be active again when the environmental conditions become suitable.

Many studies have been performed to investigate varying inoculum pre-treatment technologies and their impact on hydrogen production but most of them have used glucose as substrate. Instead, few works performed using complex substrates employed pre-treatments other than heat shock to select H_2 producer communities. The need to investigate different inoculum pre-treatment technologies using organic wastes as substrate is a crucial issue to design full scale plants. The most investigated inoculum pre-treatments either using glucose or organic wastes are discussed in the following section. Figs. 1and 2 show a summary of studies in the literature that used varying inoculum pre-treatments for dark fermentation when the substrates were glucose and organic waste respectively. Enhancement of H_2 yield (mol mol^{-1} glucose or ml H_2 g^{-1} $VS_{substrate}$) has been considered as the main criteria for the efficiency of pre-treatments in most of the studies. However, there are additional criteria that should be considered to compare deeply the different pre-treatments.

These include rapidity and duration of pre-treatment, flexibility with types and compositional variability in the inoculum or substrate, controllability of pre-treatment process and reproducibility of results, toxicity, waste disposal requirements, and long-term environment effects, costs including equipment cost and operating cost based on materials and energy consumption and finally ability to scale up. All these critical issues should be considered together with H_2 yields in order to judge if the use of a certain pre-treatment is advantageous in full scale.

2.1. Thermal pre-treatment

Thermal pre-treatment or heat shock is the most widely used method for inhibiting H_2 consumer microorganisms in anaerobic mixed microflora and selecting a culture rich in H_2 producing bacteria. Heat shock has been employed extensively as an inoculum pre-treatment using simple sugars (Chaganti et al., 2012; Pendyala et al., 2012; Shanmugam et al., 2016; Sivagurunathan et al., 2017; Yin et al., 2014). Also, heat shock has been used as the main inoculum pre-treatment method when complex wastes were used as substrate (Alibardi and Cossu, 2016; Bakonyi et al., 2014a; Kumar et al., 2015a; Liu et al., 2006). The main H_2 producers that are present in anaerobic mixed communities are from the genus *Clostridium* spp. and *Bacillus* spp. which are spore forming microorganisms. Hydrogenotropic methanogens that are the main H_2 consumers are very sensitive to heat while spore forming H_2 producers can resist high temperatures by sporulation (Zhu and Béland, 2006). Also, lactic acid bacteria whose presence is not favoured in DF due to substrate competition may be inactivated in temperatures above 50 °C (Noike et al., 2002; Kim et al., 2009).

Duration of heat pre-treatment and temperature for pre-treatment are two crucial parameters which should be optimized. Very short pre-treatment time or low temperatures may not be effective to inhibit majority of H_2 consumers while long pre-treatment or very high temperatures may lead to loss of H_2 producer's activities. According to the literature, the ranges for the temperature and duration of heating are 65–100 °C and 15 min-2 h respectively (Bakonyi et al., 2014b; Sivagurunathan et al., 2016).

There are a lot of studies which used heat shock as an inoculum pre-treatment for dark fermentative H_2 production. They used varying temperatures and pre-treatment times and obtained different results. Wang and Wan (2008) obtained a H_2 yield of

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