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A critical review on inhibition of dark biohydrogen fermentation

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ABSTRACT

Dark fermentation of organic wastes for hydrogen gas (H_2) production is an attractive strategy of being renewable and carbon neutral. However, various toxic or inhibitory compounds can significantly limit sustainable operation and widespread adoption of this biotechnology. The metabolism of fermentative microbes in dark fermentation system can be inhibited by excess substrate, micronutrients, macronutrients, metal ions, high temperature, acidic pH, un-dissociated organic acids, competitive microbes, and substrate-derived toxic substances. This review paper introduces these potential inhibitory compounds, inhibition mechanisms, and provides engineering perspectives on the control of inhibition.

1. Introduction

Development of sustainable energy is an important task to be realized in the near future. Currently, we primarily depend on various fossil fuels such as oil, coal and natural gas. However, the adequacy of worldwide reserves of fossil fuels to meet our long-term energy demand is questionable [1]. Furthermore, utilization of fossil fuels results in various adverse impacts on our environment, such as air pollution and greenhouse gas emissions. Hence, renewable, clean energy sources are essential to reduce dependency on fossil fuels and drive establishment of sustainable societies. Renewable energy is derived from non-fossil and nonnuclear sources in ways that can be replenished, are sustainable and have no harmful side effects. The ability of an energy source to be renewed also implies that its harvesting, conversion, and use occur in a sustainable manner. Renewable energy includes solar, wind, hydro, oceanic, geothermal, biomass and other sources of energy derived from "sun energy", which is thus renewed indefinitely. Forms of useable energy include electricity, hydrogen, fuels, thermal energy and mechanical energy [2].

Among the various renewable energy sources, hydrogen (H_2) is considered as one of the most preferential fuels for future, due to high-energy efficiency and cleanness. Hydrogen gas has been deemed the future fuel, and it is believed that a hydrogen- based economy would cause less pollution than a fossil fuel based economy [3,4]. Hydrogen as an energy carrier has been proven to be one of the best, the most versatile, and efficient fuels for transportation [5,6]. The combustion of hydrogen produces only water vapor without CO, CO_2, hydrocarbons or fine particles, minimizing environmental problems, and hence hydrogen as the future fuel has drawn significant attention [7,8]. Hydrogen has significant advantages as an energy resource: (a) the combustion of hydrogen gas can be pollution-free in fuel cell systems [9], (b) energy efficiency in hydrogen fuel cells is ~50%, more efficient than gasoline; thus, hydrogen fuel cells are deemed as future supply for automobiles [10], (c) hydrogen gas has a high energy yield of 122 kJ/g, and this yield is 2.75-fold greater than that from hydrocarbon fuels on mass basis [11], (d) the conversion efficiency of hydrogen to electricity could be doubled using fuel cells instead of gas turbine [10], and (e) hydrogen can be stored as a metal hydride [12].

Most of the global H_2 is now produced via steam reforming of non-renewable hydrocarbons, which also has an enormous greenhouse-gas footprint [1,13]. In contrast, various biotechnologies such as dark fermentation and photo-fermentation provide an attractive and environmentally friendly way of H_2 production from renewable sources [1,13–25]. Photo-fermentation produces hydrogen by photosynthetic microorganisms using water molecules or simple acids as electron donors, such as algae, protists and photosynthetic bacteria [26,27]. The dark fermentation is carried out by fermentative hydrogen-producing microorganisms, such as facultative anaerobes and obligate anaerobes [28,29]. Dark fermentation has several advantages over photo-fermentation. Dark fermentation can utilize various organic wastes and wastewaters as a feedstock [14–25]. Dark fermentation offers relatively higher H_2 production rate compared to photo-fermentation [16,17]. The dark fermentation process is independent of weather conditions, which provides a clear benefit over photo-fermentation [16,25]. Furthermore, compared to photo-fermentation, dark fermentation would

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be more suitable for bio-H_2 production from complex substrates [5]. Additionally, by-products (e.g. organic acids, alcohols etc.) of dark fermentation can be potentially used for the synthesis of bioplastics [18,19], as the electron donor for biological denitrification [9,10], further recovery of bio-H_2 or other value-added products using microbial electrochemical technologies or photo-fermentation [20–25]. Dark fermentative bio-H_2 production is a complex microbiological process. Microbial oxidation of organic substrates generates electrons that need to be disposed of in order to retain electrical neutrality. Carbohydrates, mainly glucose, are the preferred carbon source or electron donor for hydrogen-producing microbes, and protons (H^+) act as the electron acceptor which are reduced to molecular H_2 to balance the intracellular reducing power [30]. Furthermore, the process gives rise to acetic and butyric acids production that yields ATP. There are various types of fermentative microorganisms that can produce H_2 via dark fermentation. Strict anaerobes (e.g., *Clostridium*) are the most common class of microorganisms that produce H_2. A few facultative microbes (e.g., *Enterobacter*) have been also identified as H_2 producers in dark fermentation [31–34]. However, H_2 production in dark fermentation can be influenced by several process parameters and environmental factors including metabolic pathways, pH, temperature, substrate type, organic loading rates, microbial competition, by-products, availability of macronutrients and micronutrients [35–58]. If the aforementioned parameters are not kept within an optimum range, they may inhibit the activity of hydrogen-producing microbes. For instance, high substrate concentration is preferred for higher H_2 production rates, but significant accumulation of organic acids may trigger inhibition due to un-dissociated acids at low pH. Thus, H_2 production can be negligible in dark fermentation systems receiving a high concentration of feedstock. Moreover, organic wastes or wastewaters used in dark fermentation may initially contain various micronutrients (N, P, S), macronutrients including metal ions, other toxic substances (e.g., furanic and phenolic compounds) at concentrations that may inhibit the metabolism of hydrogen-producing microbes [35–58]. Furthermore, toxic compounds can be also produced by other microorganisms such as lactic acid bacteria during fermentation [59–62].

In the last few years, state-of-art of dark fermentative bio-H_2 production has been extensively reviewed by many researchers primarily focusing on the potential of various organic wastes as feedstock, bioreactor design, operation, and inoculum (pure vs. mixed culture) type [13,39,45–57,199]. However, commercialization of dark fermentation needs further development in terms of engineering sustainable operation and control that require a clear understanding of the various inhibitory or toxic compounds that can potentially reduce the H_2 production rate and yield. This article presents a review on various inhibitory compounds, fundamental aspects of inhibition mechanisms, and outlines the possible engineering approaches to control inhibition in dark fermentation.

2. Substrate inhibition

Higher organic loading rate is preferred for energy-efficient operation of the fermentation process to minimize energy requirements for operation (primarily heating cost). However, a minimum initial substrate concentration is also important for activating germination and to prevent re-sporulation of spore-forming bacteria [37,63,64]. Increasing substrate concentrations within an optimum range typically enhances H_2 production in dark fermentation. However, high substrate concentrations may be unfavorable to H_2 production, since the activity of hydrogen-producing microbes may be inhibited in several ways including accumulation of volatile fatty acids (VFAs), lower intracellular pH, and high H_2 partial pressure (see Fig. 1) [36,42,65–67,201]. Therefore, the optimization of substrate concentration or food-to-microorganism ratio and organic loading rate is critical to avoid substrate inhibition.

Batch studies reported that food-to-microorganism (F/M, $TCOD_{substrate}$/$gVSS_{seed}$) ratios higher than 6 may substantially decrease the H_2 yield in mixed culture fermentation of both synthetic substrates and the real organic waste [25,42]. In continuous-flow dark fermentation, organic loading rate (OLR) higher than 100 g COD/L-d typically inhibits hydrogen-producing microbes [37,68]. Table 1 presents a summary of inhibitory substrate concentrations reported in the literature. Although a wide variety of substrates have been explored for dark fermentation, most of the reported studies mainly used carbohydrate sources, such as glucose, sucrose, starch, or xylose. A few studies also used real organic wastes and wastewater as a substrate for dark fermentation. Most of the batch studies have been conducted at initial substrate concentrations of 1–50 g COD/L (see Table 1), and a majority of these studies have suggested that initial substrate concentrations above 20 g COD/L may decrease H_2 production via substrate inhibition (see Fig. 1(b)). Despite that, the inhibition threshold is not consistent, due to several factors. First, microbial inocula were very heterogeneous because the mixed-culture was mainly used for fermentation. Second, the concentrations of hydrogen-producing microorganisms and reactor configuration were different, which can also lead to different inhibitory substrate concentrations.

Optimization of influent substrate concentration would be important to avoid substrate inhibition in H_2 production. For instance, Kargi and Pamukoglu [69] showed that controlled addition of the substrate such as fed-batch (draw and fill) is effective to avoid substrate inhibition in dark fermentation. However, this approach may not be always feasible for commercial application. From engineering perspectives, it would be more feasible to design and operate the fermentation system based on the optimized F/M ratio or OLR to control substrate inhibition [25,37,42,44,48,68]. Literature suggests that maintaining higher biomass concentration in the dark bio-H_2 fermentation system may diminish substrate inhibition effects and allow the operation of fermentation systems at high OLR [37,42,44,48,69–75]. Hence, the partial recycle of the biomass from the fermentation effluent stream, membrane separation of biomass, immobilization of cells with carrier media can be a more feasible option for substrate inhibition control [37,42,44,48]. Based on these strategies, a significant effort has been devoted to developing more efficient bioreactors over conventional CSTR for the operation of bio-H_2 fermentation at higher OLR. Table 2 summarizes the performance of different advanced bioreactor configurations for biohydrogen production. Integrating a hollow-fiber membrane module into a CSTR significantly improved the H_2 yield from 1.2 mol H_2/mol $hexose_{added}$ to 1.87 mol H_2/mol $hexose_{added}$ for bio-H_2 production from tofu processing waste at 8 h HRT [70]. Dark bio-H_2 fermentation in anaerobic membrane bioreactors (AnMBRs) showed significantly higher H_2 production rates, up to 66 L H_2/L-d [44]. Granular activated carbon, plastic media, and glass beads have been extensively used as carrier media for immobilization of H_2-producing biomass in carrier-induced bioreactors such as up-flow anaerobic fixed-bed reactor, and anaerobic fluidized bed reactors [71–73]. Granular sludge based bioreactors (e.g., up-flow anaerobic sludge blanket reactor) have been also deployed to improve bio-H_2 production [74,75]. Granular sludge has been also found more resistant to substrate inhibition than dispersed or suspended biomass [76–79]. For instance, Show et al. [78] reported that granular sludge-based dark fermentation system could handle higher organic loading rates than conventional continuous stirred tank reactor (CSTR). Hung et al. [77] found that the optimization of the microbial community structure in the granular sludge would be important to enhance fermentative H_2 production, since the presence of certain aerobic microorganisms such as *Enterobacter aerogenes, Bacillus* sp., and *Bifidobacterium* sp. can stimulate the microbial activity of H_2 producers by scavenging oxygen molecules [77]. To avoid biomass washout, Hafez et al. [68] proposed an integrated dark bio-H_2 production system combining CSTR followed by gravity settling for substrate inhibition control by increasing biomass concentration; the system showed high H_2 yield of 2.8 mol H_2/mol glucose at high OLR of 103*g* COD/L-d.

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