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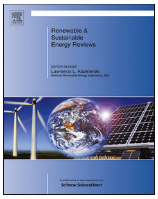

A review of sustainable hydrogen production using seed sludge via dark fermentation

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ABSTRACT

In recent years, the production of hydrogen (H_2) via dark fermentation has become increasingly popular because it is a sustainable approach to produce clean energy. This review presents an overview with a critical analysis of the technical challenges in obtaining high H_2 yield through dark fermentation. Particular focus is given to the pretreatment methods that affect H_2 production. We observed that heat pretreatment is the most frequently applied and the most effective method of eliminating H_2-consuming bacteria (HCB) while preserving H_2-producing bacteria (HPB). The pre-dominant HPB species after pretreatment belongs to the genus *Clostridium* and hence the fermentation conditions are optimized according to their preference for H_2 production. Besides, we also reviewed fermentation conditions such as substrate, pH, temperature, oxidation–reduction potential (ORP), types of nutrient and inhibitor substrate, to obtain clearer insight on the influences of critical parameters in H_2 production.

Contents

Abbreviations: H_2, hydrogen; HPB, hydrogen producing bacteria; HCB, hydrogen consuming bacteria; POME, palm oil mill effluent; UASB, up flow anaerobic sludge blanket; TVS, total volatile solid; VS, volatile solid; BES, 2-bromoethanesulfonate; MSW, municipal solid waste; VFA, volatile fatty acid

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1. Introduction

Hydrogen gas (H_2) is an alternative fuel to reduce the over reliance on fossil fuels as the primary energy used in vehicles and machines. Generally, H_2 fuel can be used in conventional gasoline engines with modifications in order to generate energy via combustion in the air [1,2]. The combustion of H_2 is sustainable and environmentally friendly because it does not generate greenhouse gases such as carbon dioxide and methane [3,4]. Hydrogen also possesses high energy yield (141.9 J/kg) among the known fuel types such as methane (55.7 J/kg), natural gas (50 J/kg), biodiesel (37 J/kg) and ethanol (29.9 J/kg) [5]. However, more than 96% of global H_2 is generated from fossil fuels [6,7]. Therefore, there is an urgency to develop a more cost-effective and environmentally friendly technology to for H_2 production.

Dark fermentation is a biological approach commonly used to produce H_2 in the absence of light [8]. This process does not require solar input and hence the configuration of the bioreactor is simpler and cheaper [9]. Most importantly, this technology has attracted attention because it can use a versatile range of substrate, particularly renewable resources that are organically rich such as stillage, sludge, leachate, pomace, stalks and bagasse [10–12]. Due to cost and environmental concerns, organic waste material is a better choice of substrates than pure compounds such as sugar or starch. This technology allows dark fermentation to be integrated into wastewater treatment systems to produce H_2 and to treat wastewater.

Seed sludge contains diverse microflora that can produce H_2 via dark fermentation [13–16]. Microorganisms found in the seed sludge are more beneficial than pure cultures because they are more adaptive to environmental stresses including limited substrates, and changes in pH and temperature. Moreover, the diverse microflora present in the seed sludge might provide synergistic interactions that improve substrate degradation and thus enhance H_2 production. Unfortunately, microflora in the seed sludge usually consists of both H_2-consuming and H_2-producing bacteria (Table 1). Therefore, it is essential to eliminate the activity of H_2-consuming bacteria (HCB) in order to increase H_2 production from H_2-producing bacteria (HPB). To achieve this, seed sludge can be pretreated using various physical and chemical pretreatment methods to enrich HPB. However, the search for the most effective pretreatment method for this purpose is still under intensive research.

Apart from the variety of HPB involved in dark fermentation, high H_2 yield is also associated with fermentation conditions including pH, temperature and types of substrate. These factors influence H_2 production by altering the physiological properties such as the enzymatic activities of HPB. In addition, H_2 production can be further enhanced by supplements or constrained by inhibitors. Theoretically, a maximum of 12 mol of H_2 is produced from 1 mol of glucose.

$$C_6H_{12}O_6 + 6H_2O \rightarrow 12H_2 + 6CO_2 \quad (1)$$

However, currently the highest reported H_2 yield is only about 20% of this maximum yield. Therefore, in order to improve H_2 yield, it is important to recognize the major contributing factors in H_2 production.

This paper critically reviews the challenges of H_2 production using seed sludge as inoculum, focusing mainly on (1) the strengths and weaknesses of different pretreatment methods on the seed sludge; and (2) the effects of different factors including types of potential substrate, operation conditions, nutrients and inhibitors, and the diverse microflora in seed sludge.

2. Factors affecting hydrogen production by seed sludge

2.1. Effects of sludge pretreatment

In order to enhance H_2 production, pretreatment is commonly used to enrich HPB. Pretreatment must be able to selectively preserve HPB while eliminating HCB. Untreated seed sludge generally produces low H_2 yield (<1.0 mol H_2/mol glucose) and pretreated seed sludge successfully improves H_2 yield (Supplementary Tables S1–S4). This is verified by the hydrogenase

Table 1
Example of H_2 producing and consuming bacteria with their characteristics.

Organisms	Functions	Characteristics	Ref.
Clostridium spp.	H_2 production	Obligate and mesophilic anaerobes The most popular H_2 producer Ferment a wide range of carbohydrates and produce H_2 E.g. *Clostridium butyricum, C. acetobutylicum, C. tyrobutyricum, C. saccharolyticum*	[17–20]
Thermoanaerobacterium spp.	H_2 production	Obligate and thermophilic anaerobes E.g. *Thermoanaerobacterium thermosaccharolyticum*	[21]
Ethanoligenens spp.	H_2 production	Obligate anaerobes Produce solvent during H_2 production E.g. *Ethanoligenens harbinens*	[22]
Bacillus spp.	H_2 production	Facultative anaerobes May possess important features such as salt tolerance E.g. *Bacillus megaterium*	[23]
Enterobacter spp.	H_2 production	Facultative anaerobes Have better tolerance against oxidative stress E.g. *Enterobacter aerogenes*	[22]
Klebsiella spp.	H_2 production	Facultative anaerobes Have better tolerance against oxidative stress E.g. *Klebsiella pneumonia*	[24]
Methanogens	H_2 consumption	Obligate anaerobes Utilize H_2 for methane production E.g. *Methanobacterium* spp., *Methanococcus* spp. etc.	[25]
Other H_2 consuming bacteria	H_2 consumption	Obligate/facultative anaerobes Utilize H_2 as electron donor and precursors for metabolic compounds E.g. *Lactobacillus* spp. and *Bifidobacterium* spp.	[26,27]