INTERNATIONAL JOURNAL OF HYDROGEN ENERGY 39 (2014) 7270–7277

Available online at www.sciencedirect.com

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journal homepage: www.elsevier.com/locate/he

Enhanced hydrogen production by a newly described heterotrophic marine bacterium, *Vibrio tritonius* strain AM2, using seaweed as the feedstock

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ARTICLE INFO

Article history:
Received 19 December 2013
Received in revised form
18 February 2014
Accepted 27 February 2014
Available online 26 March 2014

Keywords:
Vibrio
Fermentation
Hydrogen
Seaweed
Mannitol

ABSTRACT

To achieve more stable bio-hydrogen (bioH_2) production from non-food feedstocks, stable feedstock preparations of marine biomass and an efficient bioH_2 system using marine bacteria under saline conditions are two important key technologies that needed to be developed. *Vibrio tritonius* strain AM2, which was isolated from the gut of a marine invertebrate, was cultured under various conditions in marine broth (at initial 2.25% (w/v) NaCl) supplemented with mannitol, a seaweed carbohydrate, to evaluate its hydrogen production. The maximum molar yield of bioH_2 was recorded as 1.7 mol H_2/mol mannitol at pH 6 and 37 °C. The mannitol-grown cells had higher yields of bioH_2 than the glucose-grown cells in the pH range 5.5–7.5. Compared to glucose, mannitol might be a better substrate for bioH_2 production using strain AM2. Fermentation product profiling revealed that strain AM2 might be utilising the formate-hydrogen pathway for bioH_2 production. Furthermore, strain AM2 was able to produce hydrogen from powdered brown macroalgae containing 31.1% dry weight of mannitol. The molar yield of hydrogen reached 1.6 mol H_2/mol mannitol contained in the seaweed feedstock. In conclusion, strain AM2 has the ability to produce hydrogen from mannitol with high yields even under saline conditions.

Introduction

The World Energy Outlook 2013 [1] reported that the orientation of global energy sectors is currently shifting drastically, resulting in many major importers becoming exporters and former exporter countries becoming leading centres of growth in terms of global demand. Evidently, there is an urgent need for alternative global energy sources to allow us to decrease our dependence on fossil fuels, which will inevitably be depleted and whose use is implicated in environmental pollution and climatic change. Hydrogen gas (H_2) is credited as one possible candidate with several socio-economic, technological and environmental benefits [2]. The gas offers great

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http://dx.doi.org/10.1016/j.ijhydene.2014.02.164

advantages over hydrocarbon fuels because its combustion yields 2.75 times more energy (122 kJ/g) than fossil fuels without the emission of CO_2 and also because it can be directly used to power fuel cells [3,4]. In addition, this extremely clean and valuable energy carrier serves as an important feedstock in some industries. Finally, it is effective in detoxifying a wide range of water pollutants and is regularly used in the fertiliser and petroleum industries [3–5].

Rising energy costs in many countries [1] have accelerated research being performed locally and/or internationally with the aim of finding energy sources to boost the economy. H_2, a lucrative fuel alternative, is predicted to be a notable contributor to the total energy market accounting for up to 10% of the market by 2025 [6]. It is also predicted to be a viable domestic energy resource for use in all economic sectors and regions around the world [5]. However, acquiring a sustainable alternative energy source is a daunting task. Currently, the prospect of establishing a hydrogen energy economy cannot be fully realised in a sustainable way. Despite their advantages, conventional technologies for H_2 production are still heavily dependent on the reformation of fossil fuels, and most processes require intense energy at high temperatures (>850 °C) [3]. Thus, the majority of H_2 production is not conducted in a renewable and environmental friendly manner, which conflicts with the initial purpose of its application. The development of renewable technologies has become the primary bottleneck in many conventional methods of H_2 production, including the most sustainable approach, such as electrolysis [3,6,7]. For instance, the electrolysis approach suffers from two drawbacks: only approximately 65% of the energy is efficiently captured using the latest technologies, and a high electrical capacity is needed for the process to occur [8].

Currently, the best alternative seems to be biological processes, which utilise both renewable feedstock and energy [9]. Biological H_2 production via fermentation is attracting a lot of interest, predominantly because the H_2 production rate is significantly higher than that achieved by other methods. In addition, fermentation is applicable to a wide range of complex forms of organic substrates, and offers a simple system construction [4,10]. Simultaneously, rapid advances in the biofuel industry are increasing demand for an alternative feedstock that is more likely to be sustainable both economically and environmentally. In 2008, 87 GL of liquid biofuels were produced from crops intended as food production such as corn and sugarcane [11]. The output figures increased further in 2010 and have directly affected choices between various feedstocks across different regions of the world [12] to meet demand and to alleviate public concerns over the "food vs. fuel" issue. The choice has widened and now includes cellulosic biomass from agricultural residues or the food industry, carbohydrate-rich industrial wastewater and wastewater sludge [3]. With this rationale in mind, macroalgae has also recently come under consideration as an ideal biomass feedstock for bioH_2 production [13–15].

Brown macroalgae, in particular, present several advantages that complement global needs for both food and energy production [13–15]. Clearly, their marine-based cultivation has an edge over any terrestrial biofuel crop due to the lack competition for arable land, the high rate of productivity [16] and the lack of requirements for fresh water and fertiliser. Additionally, the lack of lignins and low cellulose and lipid content have made sugar extraction feasible by simple biorefinery processes such as milling, leaching and crushing [14,15]. High carbohydrate and sugar content also permit their successful conversion to various gaseous and liquid fuels. Brown macroalgae have a complex composition, and the potential breakdown of all components promises an efficient bioH_2 production. In general, alginate forms the major structural component of brown macroalgae and its utilisation, which had previously been recognised as the greatest challenge to biological ethanol production, has already been achieved by the metabolically engineered *Escherichia coli* [14] and *Sphingomonas* sp. [13]. Alternately, mannitol, the most abundant storage component, can be extracted from milled macroalgae more easily than alginate [16]. This primary stored sugar can account for 20–30% of dry weight in some *Laminaria* species [17–19] and has been successfully used for bioethanol production [16,19,20].

The backbone of a successful fermentative process is the acquisition of a good host microorganism [2]. Seaweeds are rich in minerals and are known to contain relatively more sodium (Na^+) than land vegetables [21]. At present, a high sodium (Na^+) concentration has been reported to adversely affect the H_2 productivity of *Enterobacter aerogenes* and *Clostridium* species [9,22,23]. Oren [24] and Pierra et al. [25] reported that hydrogen producing microbes in saline environments were likely to be halophilic and/or halotolerant. In another words, the halophilic and/or halotolerant natures of the host microbes can provide huge advantages in bioH_2 production in solving the "food vs. fuel" and the "water vs. fuel" conflicts. Utilization of seawater, which covers more than 90% of the available water resources of the earth, is an ideal solution in the use of seaweed as feedstock for bioH_2 production if we can find suitable marine or halophilic host microbes.

In a recent screening of effective H_2 producers from seaweed carbohydrates under saline conditions, a newly-described facultative anaerobic marine bacterium, *Vibrio tritonius* strain AM2, was isolated from the gut of sea hare (*Aplysia kurodai*), which is a herbivorous marine animal [26,27]. The vibrio showed an appropriate H_2 production in the glucose- and mannitol-supplemented batch cultures using a seawater broth without any pH controls. The bioH_2 production is an atypical characterisation in the genus *Vibrio*, but *V. tritonius* is so unique to possess the bioH_2 production pathway [27]. As vibrios are phylogenetically related to *Enterobacteriaceae* [26,28], such as *E. coli* and *E. aerogenes,* it is interesting to compare their metabolic pathways, with particular interest in the H_2-producing pathway. H_2 production by enterobacteria is commonly achieved by formate hydrogen lyase (FHL), which produces H_2 and carbon dioxide (CO_2) through formate oxidation [29–32]. The majority of studies on fermentative H_2 production have investigated *Clostridium* [33,34], as a representative of strict anaerobes and *E. coli* [35,36] and *E. aerogenes* [23,37], as representatives of facultative anaerobes. However, research focusing on H_2-producing marine vibrios has never been reported yet. In this study, the unexpectedly high H_2 productivity of marine vibrios cultured with glucose and mannitol under saline conditions is reported. Moreover, using the marine vibrio, 3 L scale batch