



Bioprocess engineering for biohythane production from low-grade waste biomass: technical challenges towards scale up

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A concept of biohythane production by combining biohydrogen and biomethane together via two-stage anaerobic fermentation (TSAF) has been recently proposed and considered as a promising approach for sustainable hythane generation from waste biomass. The advantage of biohythane over traditional biogas are more environmentally benign, higher energy recovery and shorter fermentation time. However, many of current efforts to convert waste biomass into biohythane are still at the bench scale. The system bioprocess study and scale up for industrial application are indispensable. This paper outlines the general approach of biohythane by comparing with other biological processes. The technical challenges are highlighted towards scale up of biohythane system, including functionalization of biohydrogen-producing reactor, energy efficiency, and bioprocess engineering of TSAF.

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Introduction

Huge amount of waste biomass [1] generated from various social activities such as animal wastes, food processing

wastes, agricultural residues and algal blooms, is a worldwide environmental concern. On the other hand, it contains renewable carbon and energy resources [2,3]. Value-added valorization of waste biomass is of great importance to a sustainable society [4]. Hythane, as a mixture of hydrogen and methane, also known as hydrogen enriched compressed natural gas (HCNG), has received extensive attention as a vehicle fuel [5]. Hythane displays remarkable advantages over compressed natural gas [6], such as reduced greenhouse gas (GHG) emissions, and improved fuel efficiency. One green alternative to provision of sustainable hythane instead of fossil base is two-stage anaerobic fermentation (TSAF) of waste biomass [7,8,9]. Note that much of previous knowledge about conventional TSAF is mainly focused on acidification (first stage) and methanogenesis (second), while not aiming at hydrogen production but enhanced methanogenesis [10]. In contrast, biohythane consists of hydrogen, methane and carbon dioxide harvested from the TSAF with the first stage for biohydrogen generation, which may be upgraded to biobased hythane by removing the carbon dioxide in one step [8]. Advantages of biohythane over traditional biogas are improved energy recovery, shortened fermentation time, flexible H₂/CH₄ ratio, and more environmentally benign and process robustness for handling waste biomass [7,8]. We have reviewed the research advances of TSAF for the coproduction of hydrogen and methane and proposed a new concept of biohythane in 2013 [8]. Since then, the term of biohythane has been gradually accepted in the field of gaseous biofuel [7,9,11–13,14,15–17]. However, scale up of biohythane system has yet to be developed, although a process demonstration for hydrogen and methane production from sugar-based kitchen waste via TSAF was performed [18]. Process engineering for biohythane production is still in its infant stage.

In this paper, we outline the biochemical reactions and thermodynamics of biohythane production by comparing with other biological processes for energy production from waste biomass. The technical challenges are highlighted towards scale up of biohythane production process, including functionalization of biohydrogen reactor, energy efficiency of biohythane system, and system engineering of TSAF.

Biochemical reactions and thermodynamics of biohythane

Two-stage biohydrogen and biomethane (biohythane) production was compared with other typical biofuel processes using glucose as the model substrate, focusing on thermodynamic and technical evaluation (Table 1). Most of the single-stage bioprocesses (hydrogen [19], methane [20], or ethanol [21]) face the challenges on how to deal with the remaining residuals. Volatile fatty acids (VFAs), as the main components of the fermentation residuals in anaerobic fermentation, can be further converted into energy carriers such as methane [7^{**}], hydrogen [22], electricity [20] or other biochemicals [23] by establishing a second anaerobic stage. Among all the listed pathways, the maximum bioconversion of glucose to hydrogen (12 mol/mol glucose) results in the highest theoretical energy recovery. An energy recovery higher than 100% is the result of the absorption and conversion of external heat into biohydrogen. However, these pathways are limited by the strict requirements of the substrate [24], low process efficiency, or expense of the reactors [25]. A major difference between bioethanol and biohydrogen/biomethane is that a pure strain and a narrow range of substrates are mostly needed for the former [21]. Harvesting electricity or value-added chemicals through microbial technologies is an emerging approach for waste valorization [26^{*},27]. However, the scale up of MFC for practical application still suffers from the cost-intensive materials, and long-term operation stability [28].

Given that a hydrogen yield of 4 mol/mol glucose can be achieved through dark fermentation, theoretical energy recovery for hydrogen production (41%) is the lowest value among the biofuel processes (Table 1). In fact, the current hydrogen yield is normally lower than 2 mol/mol glucose due to the limited metabolic fluxes [29]. Single-stage hydrogen production through dark fermentation is thus not energy and cost effective [19], and should be combined with other value-added processes. Methane fermentation has been well developed, which is, however, time-consuming and challengeable for treating high-solid organic waste. Instead, the biohythane system via TSAF resulted in enhanced energy recovery and reduced fermentation time [7^{**},8^{**}]. In addition, in a biohythane system dealing with lignocellulosic biomass, saccharification and biohydrogen production could be simultaneously implemented in the first stage via microbial consortium engineering [30].

Technical challenges for scale up Microbial consortium and engineering control of biohydrogen and biomethane processes

The traditional anaerobic methane fermentation normally incorporated microorganisms with different functions to establish a synergic microbial consortium [31]. In particular, two kinds of bacteria take part in the methanogenesis process: one responsible for the conversion of

acetic acid to methane, and the other for the reaction of carbon dioxide and hydrogen into methane. In order to harvest hydrogen from the overall process and generate biohythane, the hydrogen-to-methane pathway has to be inhibited [16]. Three aspects are most influential: (1) microbial physiological characteristics. Most hydrogen-generating microbes other than methanogens can produce spores in stress. Different pretreatment methods could be adopted to screen hydrogen producers [32]. In general, the most common pretreatment is heat treatment and pH shock. However, some studies reported the invalidity of such pretreatment [33], because not all hydrogen-producing bacteria are directly associated with the ability to form endospores. In addition, there are also many hydrogen-consuming bacteria that can form spores, such as acetogens, certain propionate and lactate producers [34]; (2) pH control. pH control is an important strategy for continuous operation of biohythane system, where pH varies depending on microbial species and activities, feedstock characteristics, organic loading, reactor structure, temperature, etc. The difference of pH is due to various microbial reactions involved, whereas the pH influences the distribution of respective metabolic products [35]. Low pH is one of the most critical strategies to inhibit the activity of methanogenesis. The suggested optimal pH for biohydrogen production ranges from 5.0 to 6.5, whereas the neutral pH is beneficial for methanogenesis; (3) growth rates of microbes. From the perspective of thermodynamics, changes of Gibbs free energy during hydrogen production were much larger than those of methanogenesis (Table S1). This means faster rates for microbial growth in biohydrogen fermentation. On the basis of this characteristic, a number of bioprocess parameters, such as hydraulic retention time (HRT) [34], temperature [36], oxidation-reduction potential (ORP) can be manipulated to enable microbial hydrogen process to be feasible in continuous operation. For instance, shortening HRT has been frequently used to wash out the methane producers in biohydrogen stage, further contributing to the two-stage separation [34].

Functionalization of biohydrogen reactor

From the perspective of microbial metabolisms, biohydrogen is an intermediate of biomethanation and can only be harvested through inhibiting or inactivating hydrogenotrophic methanogenesis. The performance of the biohydrogen-fermentation stage directly impacts the production of biomethane and formation of fermentation residues. The feedstock type and microorganism species contribute most to the functionalization of biohydrogen reactor [37]. For instance, sugar-rich substrates are ideal for hydrogen production considering the metabolic pathway of biohydrogen [8^{**},38]. In comparison, protein-rich biowastes, such as animal manure is less desirable due to the limited hydrogen donor [38]. Cellulosic feedstock is also difficult because of its recalcitrance for microbial transformation [37]. Codigestion strategy could

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