



Lignocellulose biohydrogen: Practical challenges and recent progress



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ABSTRACT

The objective of this work is to provide update information about the recent progress on lignocellulose hydrogen conversion via dark fermentation. Therefore, in this review, the most important fields associated with lignocellulosic hydrogen fermentation are covered, firstly describing the problems associated with raw materials, followed by the discussion of pretreatment and hydrolysis methods assisting to achieve efficient hydrogen fermentation. Afterwards, issues related to fermentation inhibitors as well as advanced, integrated bioprocesses for hydrogen production purposes will be presented. Additionally, the paper gives future perspectives of lignocellulose biohydrogen.

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1. Introduction

Hydrogen is widely taken into account as a potential solution for sustainable energy generation not exclusively but especially for transportation. Driven by the advantages of using hydrogen as a new age fuel – such as only water vapor is released when it undergoes combustion – a world-wide progress has been started with the ultimate objective of promoting the transition into hydrogen based economy [1].

Nowadays, various countries and several leading companies of the automotive industry are putting efforts and investing significant amount of money to establish national hydrogen fueling

infrastructures and to produce highly energy efficient as well as environmental-friendly fuel cell vehicles [2]. In other words, hydrogen economy is not only a fine-sounding and theoretical concept anymore. However, it is important to note that the path towards its realization is still challenging due to obstacles such as the large-scale unavailability of ecologically viable production technologies.

Currently, most of hydrogen used in different industrial applications or transportation is delivered from non-renewable, carbonaceous substances e.g. methane [3]. Therefore, these energy-intensive and polluting methods do not indeed ensure “green” hydrogen. Hence, to meet the requirements of sustainable development, hydrogen should be originated from renewable resources via environmentally benign and energetically less demanding techniques. For these purposes, attention is paid to the biotechnological approaches aided by specific microbial catalysts that

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convert biomass derived materials into molecular hydrogen gas under anaerobic conditions [4].

Anaerobic hydrogen fermentation was first time reported in the late 1970 [5] and research of this area has been considerably expanded. As a result of the extensive investigations in the last 30–40 years, noticeable advancements were accomplished that have brought the term “biohydrogen” to the spotlight of public awareness. Currently, among the various conventional ways of generating biohydrogen such as biophotolysis, photo fermentation and dark fermentation, the latter appears as the most feasible one from practical point of view [4,6]. In recent decades, overwhelming projects have focused on numerous but equally substantial aspects of dark fermentative hydrogen production, including strain selection and development [7,8], bioreactor engineering [9–11], downstream processes such as biohydrogen recovery and purification [12–14], processing fermentation effluent [15] and feedstock utilization [16–18]. The latter factor, properties and valorization of fermentation raw materials such as biomass, which is in the scope of this paper, play a crucial role in biohydrogen production. It has been demonstrated well that renewable biomass can be a potential input material for hydrogen evolution prominently because of its carbohydrate-rich feature [15–17].

Biomass may be distinguished into various categories [19]. The so-called “first generation biomass” encompasses mainly food plants such as corn, wheat, sugar beet, sugar cane, etc. having easily accessible carbohydrate (e.g. starch) content. Even though these materials would be ideal from technological standpoint, their scaled-up employment for hydrogen fermentation likely fails due to the “fuel vs. food” debate. Consequently, scientists and engineers rather tend to apply “next generation organic matters” such as lignocelluloses for hydrogen bioproduction. These abundant materials are recognized as excellent sources for a range of fermentable sugars, which, unlike in case of first generation biomass are in a complex form and hardly digestible [20,21]. Thus, usage of such substrates consisting of cellulose, hemicellulose and lignin needs careful process design so as to achieve sufficient hydrogen production capacities. The most substantial stages associated with lignocellulosic biofuel generation are as follows [22]:

- Pretreatment of the raw material, when the aim is to break the recalcitrant heteropolymeric structure of lignocellulose complex.
- Hydrolysis of the cellulose and hemicellulose fractions liberated in order to attain large quantity monomeric sugars.
- Conversion of monomeric carbohydrates into biofuel by efficient microorganisms in adequate bioprocesses.

The present article attempts to survey the recent advancements made on the above-referenced crucial steps of microbiologically-assisted lignocellulose hydrogen technology. Firstly, the inherent traits of lignocellulosic biomass making it difficult to process are introduced along with pretreatment and hydrolysis considerations. Afterwards, the strategies for higher performance second generation biohydrogen fermentation will be discussed, including the mitigation of fermentation inhibitors as well as integrated fermentation concepts by touching bioreactor design, strain selection and biocatalyst improvement via metabolic engineering. Finally, possible future perspectives are assessed, as well. Writing this review paper was driven by the motivation of providing up to date information to the scientific audience about the current status and technological developments of lignocellulose biohydrogen production.

2. Characteristics of lignocellulose biomass – the roles of pretreatment and hydrolysis

Lignocellulosic biomass is the most abundant raw material in the nature, which covers hardwood, soft wood, grasses, agricultural and

forestry residues as well as secondary biofuel wastes. The annual worldwide yield of lignocellulosic biomass residues is estimated to exceed 220 billion tons. These materials are composed of holocellulose (referring to cellulose and hemicellulose together and representing up to 70–80% of lignocellulosic biomass), which is coated and intimately associated with lignin [23,24]. The former is the desired portion for hydrogen production that is built up from long-chain biopolymers, containing exclusively glucose in case of cellulose and a variety of hexoses and pentoses (i.e. xylose) for hemicellulose, respectively. On the other hand, presence of lignin is disadvantageous from the viewpoint of biohydrogen fermentation since it has notable persistency for biodegradation and only limited number of species possesses capability for its decomposition. Hence, due to the protected structure of raw cellulosic biomass, attempts on its direct conversion into hydrogen are accompanied by relatively low performances [25].

Therefore, in many cases when lignocellulosic organic matter is to be utilized, pretreatment methods seem to be necessary. The aim of any pretreatment is to increase the accessibility towards the carbohydrate-rich holocellulose. Pretreatment techniques may be carried out in versatile ways as detailed later on. Nevertheless, all of them take an effect through the elimination of the “glue” existing in between cellulose, hemicellulose and lignin, meaning that the intermolecular or interpolymer linkages have to be cleaved [26]. In addition, the common goal of lignocellulose pretreatment is to provide increased surface area, porosity and reactivity so that it could help the subsequent steps of polysaccharide hydrolysis and fermentation where the H_2 producers are involved. In recent decade, tremendous investigations have been performed with the purpose of developing feasible – both conventional and unconventional – methods for lignocellulosic biofuel technology [21,27]. Pretreatment usually starts with mechanical curing such as milling and grinding that can be followed by chemical, physicochemical, thermal, biological, etc. procedures to further destruct the tough structure. This may be achieved by diluted or concentrated acids such as sulfuric acid, hydrochloric acid, nitric acid [28] or organic acid e.g. acetic acid [29]. Alternatively to acid treatment, soaking of lignocellulosic biomass in alkali solutions e.g. NaOH, ammonia, $Ca(OH)_2$ could be used [30–32]. Chemical pretreatment may apply conventional organic solvents, novel ionic liquids [33,34] and powerful oxidizing agents such as ozone [35], while the biologically-assisted way exploits the lignocellulose-unlocking potential of fungi [33,36,37]. Besides, (hydro)thermal treatment [38], steam-explosion [39], ultrasonication [34,40] and microwave irradiation [41,42] and their combinations are also proven methods to structurally destroy lignocellulosic materials. General factors apparently influence pretreatment efficiency are the contact time, temperature and pressure applied, pH, concentration of chemicals, biomass to chemical ratio, etc. (Table 1). Moreover, it has been recently demonstrated that even if various lignocellulosic feedstocks reflect similar compositions and are pretreated under identical conditions, there could be observable difference in their fermentability into $bioH_2$ [28]. Consequently, it is assumable that even though the biomass used for a long time is routinely collected from the same source or place, the fine-tuning of the pretreatment process may be needed over the time because of the seasonal alterations in the composition of the raw material. Although pretreating substrate can play a key role to get rid of lignin sealing in order to enhance microbial hydrogen conversion, it consumes energy and therefore has a negative contribution to the whole process economy [43]. Hence, considerable attention is paid to environmental-friendly biological approaches as alternatives to mature chemical technologies since they possess the benefits of no inhibitory compound formation, mild operational conditions as well as low energy demand [36].

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