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Hydrolysates of lignocellulosic materials for biohydrogen production

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Lignocellulosic materials are commonly used in bio-H_2 production for the sustainable energy resource development as they are abundant, cheap, renewable and highly biodegradable. In the process of the bio-H_2 production, the pretreated lignocellulosic materials are firstly converted to monosaccharides by enzymolysis and then to H_2 by fermentation. Since the structures of lignocellulosic materials are rather complex, the hydrolysates vary with the used materials. Even using the same lignocellulosic materials, the hydrolysates also change with different pretreatment methods. It has been shown that the appropriate hydrolysate compositions can dramatically improve the biological activities and bio-H_2 production performances. Over the past decades, hydrolysis with respect to different lignocellulosic materials and pretreatments has been widely investigated. Besides, effects of the hydrolysates on the biohydrogen yields have also been examined. In this review, recent studies on hydrolysis as well as their effects on the biohydrogen production performance are summarized. [BMB Reports 2013; 46(5): 244-251]

INTRODUCTION

Hydrogen as one of the most abundant elements in the universe is regarded as one of the most promising alternative energy carriers as a viable energy option without CO_2 emissions and received much favorable attention. Currently, most H_2 is produced from water electrolysis or catalytic reforming of non-renewable sources such as oil, natural gas, and coal (1). These traditional methods can result in excessive energy consumption, and even cause severe pollutants emission into environment. As such, the sustainable energy development urgently demands an energy-saving and clean H_2 production technology. H_2 production from renewable biomass by dark fermentation or photofermentation is one of the ideal solutions to this problem, which is environmental friendly and less energy intensive as compared to conventional thermochemical and electrochemical processes, as it can offer the sustainable supply of usable H_2 from a variety of renewable resources with low pollution emission, low costs, regeneration and high efficiency (2, 3). So far, sugars and starch have been responsible for the major feedstock of the produced bio-H_2. However, these pure carbonhydrates are expensive for H_2 commercial application. Instead, great amounts of H_2 can be produced from cellulosic materials by fermentation under conditions that are in favor for H_2 producer and inhibit methane-producing bacteria (4).

Although these lignocellulosics biomass in nature is by far the most abundant raw material and can be converted to H_2 by hydrolysis and downstream fermentation, lignocellulosics biomass has to be converted to monosaccharides or other low-molecular-weight compounds by pretreatment and hydrolysis prior to fermentation process (Fig. 1) (1, 3, 5). In general, the hydrolysate components are diverse due to the complex structure of lignocellulose, in which glucose and xylose are the main products in the hydrolysates of lignocellulosic materials, other sugars such as arabinose may be formed in a low amount. Furthermore, some inhibitors which can decrease hydrolysis efficiency and H_2 production performance are yielded during the pretreatment and enzymolysis. Thus, the choices of pretreatment and enzymatic hydrolysis conditions doesn't only regard the overall compatibility of feedstocks, enzymes and organisms to be applied in order to increase the reducing sugar yields, but also consider effects of the hydrolysates on bio-H_2 production performance in order to improve the conversion efficiency.

HYDROLYSATES FROM PRETREATED CELLULOSIC MATERIALS

Generally, lignocellulosic biomass consists of 40-50% cellulose, 25-30% hemicellulose, 15-20% lignin, and and other extractable components (6, 7). Cellulose is a linear syndiotactic (alternating spatial arrangement of the side chains) polymer of β-d-glucopyranose units linked together by β-1,4-glycosidic bonds. The basic repeating unit of the cellulose polymer is glu-

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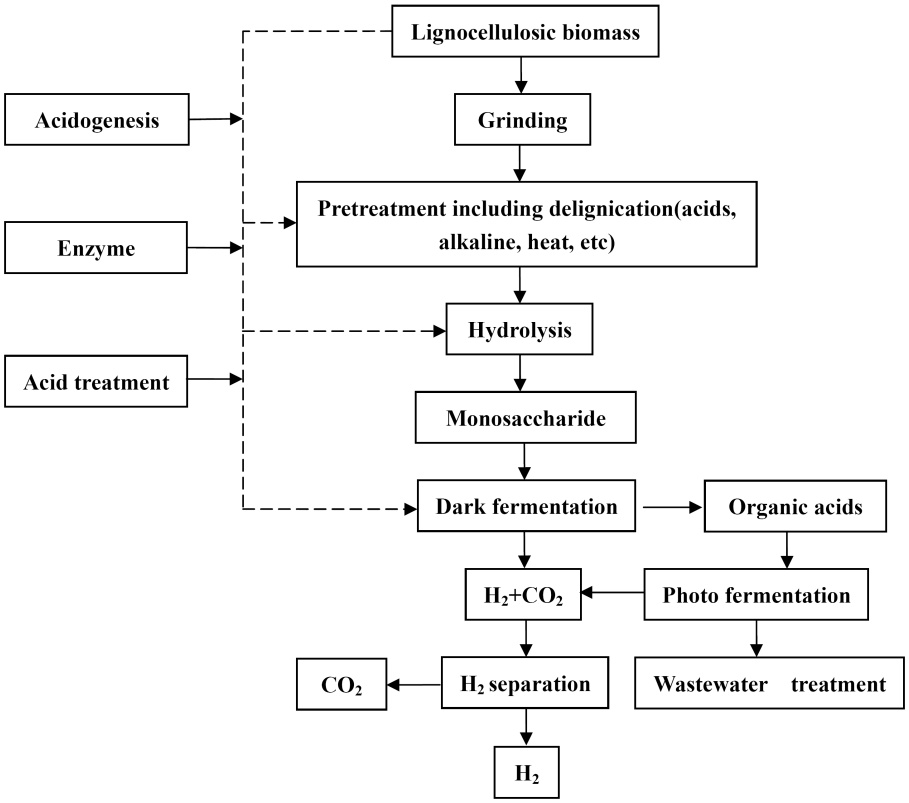


Fig. 1. A schematic diagram for biohydrogen production from lignocellulose materials.

cose anhydride which is formed via the removal of one water molecule from two glucoses and polymerize into long cellulose chains with 5,000-10,000 glucose units (8). Hemicellulose exhibits lower molecular weights than cellulose and is composed by d-xylose, d-glucose, d-galactose, d-mannose, l-arabinose, d-glucuronic acid, and 4-O-methyl-d-glucuronic acid units. Compared to cellulose, the number of repeating saccharide monomers in hemicelluloses is only 150 and the most important monomer is xylose. Lignin is a complex aromatic macromolecule formed by radical polymerization of phenyl-propane alcohols (p-coumarilic, coniferilic, and synapilic). The ratio of these components varies in different plants, tissues, and cell wall layers. The basic chemical phenyl-propane units of lignin (primarily syringyl, guaiacyl, and p-hydroxy phenol) are bonded together to form a very complex matrix (2, 9).

The cellulose chains are packed into microfibrils which are stabilized by hydrogen bonds (6). These microfibrils are attached to each other by hemicelluloses and amorphous polymers of different sugars as well as other polymers and covered by lignin, forming ordered tertiary structure with high molecular weight. These tertiary structures are often associated in the form of bundles or macrofibrils in which the individual microfibril is packed so tightly that not only enzymes but even small molecules like water cannot enter the complex framework. It indicates that both hemicellulose and lignin provide a protective sheath around the cellulose, thus the sheath must be removed or destroyed prior to effective utilization of the embedded polysaccharides (10).

The primary objectives of pretreatments are to remove or alter hemicellulose and/or lignin, to increase the pore volume and the internal surface area and decrease the degree of polymerization and crystallinity of lignocellulose, finally increasing the reducing sugar yields of hydrolysates (11). The ideal pretreatment process should achieve high yields of fermentable reducing sugars, avoiding degradation or loss of reducing sugars and the formation of inhibitors to the subsequence fermentation, thus, improving the subsequent cellulose hydrolysis in terms of minimal energy, chemicals and capital equipment use (12, 13). Currently, the often used pretreatment methods are diverse, including heat, alkaline and acid, as well as their integration. Pienkos and Zhang (14) considered that steam explosion as a heat pretreatment resulted in two separate phases: (a) an aqueous phase (hydrolyzates) containing mostly xylose and some glucose, mannose, arabinose, and galactose solubilized from hemicellulose and (b) a wet solid fraction (lignocellulose) enriched in lignin and cellulose. Cao *et al.* (15) found that dilute NaOH solution autoclaving and H_2O_2 immersing pretreatment was the most suitable method for sweet sorghum bagasse pretreatment. The highest cellulose hydrolysis yield and total sugar yield of enzymolysis were 74.29%

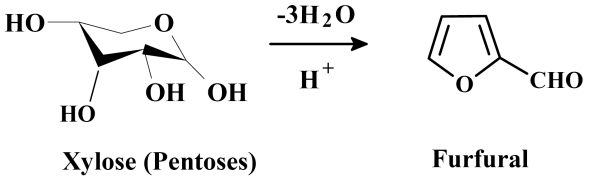
and 90.94 g sugar/100 g dry matter, respectively, which were 5.88 and 9.54 times higher than the control. Other pretreatment methods such as microwave, ionic liquids, ammonia fiber explosion, SO_2-catalyzed steam pretreatment and biological treatment considered as effective methods decomposing lignocellulosic materials had been tried. It should be pointed out that the type and concentration of monosaccharides evolved by hydrolysis are different via different materials and pretreatment methods. In general, 41.0-43.4% of glucose, 14.8-20.2% of xylose, 2.7-4.5% of arabinose, 1.8% of mannose and 0.4% of galactose can be obtained in straw hydrolysate (16), while Ibbett *et al.* (17) obtained the proportion of monosaccharides in hydrolysates from the cellulosic material heated to 200°C: arabinose 10.0%, galactose 2.9%, glucose 11.6%, and xylose 75.1%.

Furthermore, regulatory factors during enzymatic hydrolysis of the pretreated cellulosic materials are very important to hydrolysate production. These factors mainly include substrates, cellulase activity, reaction conditions (temperature, pH, etc.) and end product inhibition (cellobiose and glucose). For example, Hodge *et al.* (18) found that the rates and extents of enzymatic hydrolysis of corn stover pretreated by dilute acid declined with increasing slurry concentration, and high sugar concentrations was the primary cause of performance inhibition, except for the high viscosity and uneven slurry distribution. While at an excessive reaction conditions, parts of the monosaccharides and soluble lignin fragments are degraded or transformed into inhibitive compounds such as 2-furaldehyde (furfural) and 5-hydroxymethyl-2-furaldehyde (HMF); aliphatic acids such as formic, acetic and propionic acid; and phenolic compounds. These inhibitors may interfere with maintenance functions or osmotic pressure of cells, even directly inhibit fermentation pathway (19). The type and level of inhibitors are determined by the biomass substrate, pretreatment process and enzymolysis conditions. Ibbett *et al.* (17) found the inhibitor concentrations in hydrolysates from straws were soluble lignin 2.6%, furans 0.5%, and organic acids 5.1%.

Furfural and HMF are key degradation products of monosaccharides under thermal and acidic conditions. Furfural, which may react further to yield formic acid or may polymerize, is produced by dehydration of a fraction of the liberated pentoses from the hydrolysates of the xylose-rich hemicellulose materials (Fig. 2A) (19, 20). The dehydration of pentoses to furfural involves irreversible formation of enediol intermediates. Researches showed that furfural concentration in liquid phase increased with rise in pretreatment temperature, acid concentration or pretreatment time for tested cellulosic materials, along with decreases of glucose and xylose (21, 22). While the dehydration of hexoses such as glucose releases HMF, which can be further converted to levulinic acid and formic acid (Fig. 2B) (19, 23).

Another inhibitory substance in hydrolysates is organic acids, such as acetic acid which is formed due to the hydrolysis of acetyl groups linked to the heteropolysaccharides in hemicellulose. It can penetrate the cell membrane in undissociated form and inhibits products production through chemical interference, causing pH imbalances at high concentration and eventually cell growth inhibition or death (24, 25). However, acetic acid does not necessarily play the decisive role in causing the difference in fermentability, since some strains can directly utilize acetic acid as substrate for bioconversion such as H_2 production (26, 27). In addition, formic acid and levulinic acid may be evolved in hydrolysates and even cause the particularly poor fermentability of the hydrolysates. Meanwhile, a portion of the lignin can be solubilized into a complex mixture of low molecular weight or "monomeric" phenolic compounds during pretreatment especially acid impregnation (6).

(A) Acid-catalyzed dehydration of xylose (pentose) to furfural



(B) Acid-catalyzed dehydration of glucose (hexose) to levulinic acid and formic acid with HMF as intermediate

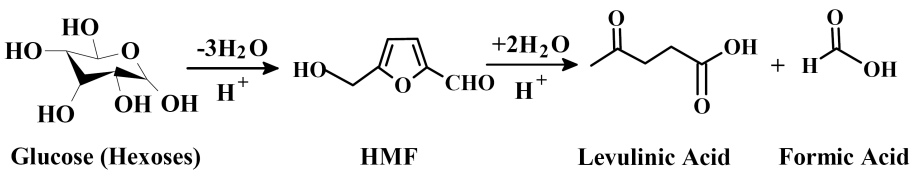


Fig. 2. Formations of furfural and HMF from monosaccharides. (A) Acid-catalyzed dehydration of xylose (pentose) to furfural, (B) Acid-catalyzed dehydration of glucose (hexose) to levulinic acid and formic acid with HMF as intermediate.

Phenolic compounds are widely known to be toxic to microbial cells because they increase membrane fluidity, consequently, affecting membrane permeability and inducing a longer acclimation of mixed cultures.

In short, the lignocellulosic material can be hydrolyzed by acid, alkali, heat, or enzymatic methods, but inhibitors, such as furfural, acetate, and 5-hydroxymethylntation, could also be produced easily. Therefore, severe parameters must be carefully optimized in the pretreatment process and some new methods should be tried to solve this problem, and a detoxification treatment of the hydrolysates to remove the inhibitors and enhance H_2 production rate is necessary.

H_2 PRODUCTION FROM HYDROLYSATES OF CELLULOSIC MATERIALS

Theoretically, 1 g of cellulose can yield 567 ml H_2 (28). However, the practical yields to direct fermentation of cellulosic materials are very low due to complex structure of lignocellulose. In this conversion process, H_2 production and bacteria growth, together with intermediates, are influenced by pretreatment conditions and type of cellulosic materials, fermentation conditions. Researches found that the lag phase of cell growth was prolonged with the increase in pretreatment severity of corn stover, even a significant decrease in the amount of H_2 produced due to a loss of total sugars and the inhibitory levels of furfural, HMF and other potential phenolics contaminants (29, 30). Chu *et al.* (31) revealed that microbial growth, colony and biofilm formation rates, higher cellulose affinity and enzymatic activity, together with beneficial synergies between different inoculum groups, were the important factors causing different H_2 production abilities, except cellulosic materials, by using the milled wheat stalk as anaerobic dark fermentation materials. Furthermore, volatile fat acids (VFAs) such as acetate and butyrate are the main products during H_2 production by dark fermentation.

Lately, many works on H_2 production have been done by using hydrolysates of lignocellulosic materials as fermentative substrate, this definitely enhances H_2 production yield. As aforementioned, hexose (C_6) and pentose (C_5) are the predominant components in the cellulosic hydrolysates. Among hexose, glucose is the main product, while the pentose fraction mainly consists of xylose. H_2 production from hexose and pentose pass through different metabolic pathways. Theoretically, the maximal H_2 yield of glucose by fermentation is 4 mol H_2 per mol glucose. However, the practical yield is low due to the intermediate formation such as acetate and/or butyrate.

As for pentose, when acetate is produced as a by-product, xylose and arabinose can be converted to H_2 with a yield of 3.33 mol H_2 per mol substrate, respectively. If butyrate is the by-product, a lower yield of 1.67 mol H_2 per mol substrate can be obtained (32). However, pentose is an unfavorable carbon substrate for H_2 production. So far, only a few pentose-fermenting microorganisms have been identified, compared with hexose-fermenting bacteria. Kádár *et al.* (33) found extreme thermophile *Caldicellulosiruptor saccharolyticus* could utilize xylose as substrate for cells growth and H_2 production by anaerobic fermentation, and observed when using a mixture of xylose and glucose as carbon source, xylose, together with glucose, was consumed simultaneously and the consumption efficiency was higher than xylose as sole carbon source. In addition, other sugars in hydrolysates such as arabinose and rhamnose can't be effectively utilized for H_2 production. Thus, further isolation of the multi-sugar fermenting microorganism is necessary to enhance the lignocellulosic hydrolysate utilization.

Obviously, H_2 production from hydrolysates of cellulosic materials is influenced by the used material and pretreatment conditions. Pattra *et al.* (22) indicated that acid hydrolysate of sugarcane bagasse was suitable for producing hydrogen by *C. butyricum* due to its high sugar concentration (glucose, xylose, arabinose) and low growth inhibitors concentrations (HAc and furfural). Pattanamanee *et al.* (34) also investigated the H_2 photo-fermention production from the hydrolysate from oil palm empty fruit bunch pretreated by sulfuric acid and obtained H_2 production rate of 22.4 ml H_2/l/h and specific hydrogen production rate of 7.0 ml H_2/g (xylose + glucose + acetic acid)/h. Lay (35) found that increased concentration of cellulose from 12.5 g/l to 50 g/l gave a lower yield from 2.18 mmol/g cellulose to 0.42 mmol/g, respectively. In view of reaction condition, Liu *et al.* (36) considered that a higher temperature resulted in a higher conversion of cellulose to H_2. Although many works on H_2 production from hydrolysates have been done, the most suitable raw materials, pretreatment methods, bacterial cultures, operating conditions, cultivation types, operating modes and processing schemes are yet to be determined for an effective and economically viable H_2 production.

Furthermore, the total sugar concentration of hydrolysates significantly gives an effect on the fermentation process. Increase in the total sugar concentration to an optimal level means an increase in H_2 production. Kargi *et al.* (37) performed effect of initial total sugar concentrations varying between 5.2 and 28.5 g/l on H_2 production by thermophilic dark fermentation. The results indicated that H_2 yield and specific H_2 production rate, together with the total volatile fatty acids (VFAs) concentration, could increase with the increase in the initial total sugar concentration (38). Certainly, fermentative conversion of cellulosic hydrolysates into H_2 is always accompanied with formation of a variety of soluble metabolites. The predominant pathway of H_2 production from reducing sugar of hydrolysates is the acidogenic pathway over solventogenesis. An excessive sugar concentration can cause accumulation of more VFAs in medium and lead to a sharp decline of pH of fermentation medium, thus, the growth and activities of hydrogen producer may be inhibited. In this process, acetate, butyrate, formate, and ethanol are the major products, followed by lactate and propionate (31, 39).

Recently, sequential dark and photo-fermentation of organic

compounds has been proposed and been considered as a promising method to improve H_2 production performance (40). During dark fermentation, sugars are converted to H_2, CO_2 and short-chain organic acids with a theoretical maximum hydrogen yield of 4 mol of H_2/mol of hexose sugar. Following, the dark fermentation effluent centrifuged and sterilized is transported into the photo-bioreactor for H_2 production by photofermentation. The obtained productivity and yield of H_2 can be significantly improved due to the reutilization of short-chain organic acids by PSB at the expense of light energy. Other methods such as temperature-shift method have also been tried to enhance H_2 production by fermentation. When temperature-shift method is adopted, it can cause higher reducing sugar production with the presence of large quantity of glucose, xylose and cellobiose in the hydrolysates, thus, a higher H_2 production rate in the fermentation can be achieved (39).

In addition, H_2 production and hydrolysates utilization are affected by initial and final pH, temperature, feedback inhibition by end-products, as well as other control parameters.

EFFECT OF INHIBITORS OF HYDROLYSATES ON H_2 PRODUCTION

The fermentation inhibitors from the lignocellulosic hydrolysates can be sorted into the following three groups (41). The first inhibitive group is organic acids such as acetic acid and formic acid, whose toxicity was interpreted as penetration of the undissociated acid into the cell and dissociation of acid at higher intracellular pH value. The second group includes furfural, 5-hydroxymethyl furfural, laevulinic acid, and humic substances, which is the by-products for sugar degradation. The third group of inhibitors is the lignin degradation products. This group of inhibitors includes a wide range of aromatic and polyaromatic compounds.

Currently, these inhibitive organic acids have been demonstrated to be used as substrate for the bacterial growth and H_2 production, which offers the advantage of combining organic wastes disposal with the production of a clean fuel (42). Specially, phototrophic fermentation by PSB has been widely used to further convert these organic acids to H_2 with light illumination (43). These organic acids include acetate, lactate, malate, succinate, benzoate, butyrate and propionate, which are utilized by different metabolic pathways during H_2 production (44). Among these different organic acids, acetic acid is a typical substrate as electron donor during H_2 production. Boran *et al.* (45) confirmed that at low light intensities and low temperatures, the acetic acid could be utilized for biosynthesis, growth and H_2 production. Evolved gas contained 99% H_2 and 1% CO_2 by volume and the overall hydrogen yield was 0.6 mol H_2 per mol of acetic acid fed. However, propionate is known to have a negative effect on the efficiency of H_2 production. High H_2 yields are associated with a mixture of acetate and butyrate fermentation products, while low H_2 yields are with propionate and reduced end products. Cheng *et al.* (46) and Zhang *et al.* (47) reported a negative correlation between propionate and H_2 yield in mixed cultures and observed that an increase of H_2 yield was concomitant with a decrease in propionate concentration. Compared to other organic acids, malate, lactate and succinate are easily utilized by PSB for H_2 production, because these acids are metabolic intermediates of glycolysis pathway. Tsygankov *et al.* (48) obtained 75% H_2 yield by *Rhodobacter sphaeroides* RV when succinate was used as carbon source.

On the other hand, furan derivatives act as strong inhibitors of many soluble enzymes involved in glycolysis and exert a large negative influence on H_2 production of bacteria compared to sodium acetate. Investigation found that bacterial cells seem very sensitive to furfural, HMF and syringaldehyde, and the inhibition of furfural to the growth and H_2 formation is more obvious than that of HMF, thus, the lag phase in furfural-added cultures was twice as long as in HMF-added cultures (49). The addition of furan derivatives (furfural and HMF) can lead to the highest inhibition of H_2 production, with a dramatic drop in H_2 yields. This can be attributed to the disturbance of membrane integrity of microorganism, and to a high valerate levels. The inhibitory effect extent depends on the inhibitor concentration. However, the actual concentrations of furfural and HMF in hydrolysates or fermentation medium are relatively low. Panagiotopoulos *et al.* (50) found that the inhibition of hydrolysates from corn stalk pretreated by mild-acid could be due to the presence of HMF and furfural during fermentation. However, the concentrations of HMF and furfural were far lower than the concentrations of 1-2 g/l resulting in less severe H_2 production inhibition. This implies that the observed inhibition can not be fully explained by the effect of HMF and furfural. Both furfural and HMF (at 1 g/l) have ever been reported to stimulate the growth of *Clostridium beijerinckii* BA101 as well as the production of acetone-butanol-ethanol through non-H_2-producing pathways (51). *Clostridium sp* is currently found to be more resistant to the inhibitors, making this strain as an ideal candidate for H_2 production from hydrolysates of lignocellulosic biomass.

Aromatic compounds such as vanillin and syringaldehyde are another type of inhibitors from degradation of lignin. Research indicated that the lag phase time as added phenol was longer than added other inhibitors and has been suggested to exert a considerable inhibitory effect in the fermentation of lignocellulose hydrolysates (49). It can be explained by the intrinsic abilities of these compounds to penetrate cell membranes: the higher the molecular mass, the slower the introduction into a cell and the shorter the lag phase. Furthermore, lignin largely contributes to the decrease in H_2 production and yield, which can be explained by their physicochemical properties, e.g. hydrophobicity, ramification, and methoxylation. de Vrije *et al.* (52) demonstrated an inverse relationship between lignin content and the efficiency of enzymatic hydrolysis of polysaccharides. High delignification caused high hydrolysis efficiency, as a result, high H_2 production ability ach-

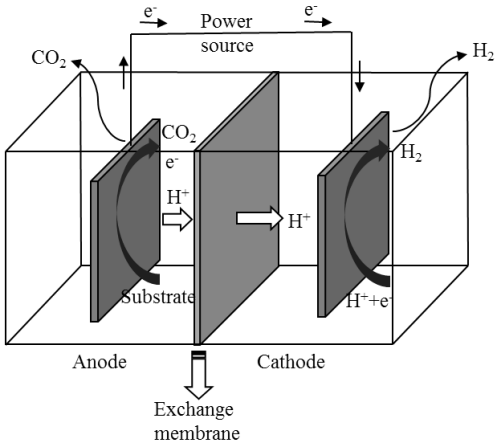


Fig. 3. Schematic illustration of bio-H_2 production by MEC with two chambers.

ieved.

In short, the representative inhibitors presented in hydrolysates have the synergistic effect on growth and H_2 production. To increase the efficiency of bio-H_2 production process from hydrolysates of cellulosic materials, the inhibitory compound concentration in the raw hydrolysates must be reduced to the levels which don't interfere in the metabolism of H_2 production.

ENHANCEMENT METHODS FOR H_2 PRODUCTION PERFORMANCE

Effective removal of inhibitors in the process of lignocellulosic hydrolysis can significantly enhance H_2 production. Although many control technologies to decrease inhibitor formation during pretreatment and hydrolysis have been adopted to enhance the biological conversion of lignocellulosic biomass, it is difficult to select the most efficient process or method due to very complex hydrolysate compositions of lignocellulosic substrate. In general, a combination of physical or chemical method with biological process or other process focused on the pretreatment of lignocellulosic feedstocks can increase the digestibility and obtain more reducing sugar, as a result, improving H_2 production. Elbeshbishy *et al.* (53) evaluated the effects of the food waste though different pretreatments on biohydrogen production in batch culture and the highest hydrogen yield of 118 ml/g $VS_{initial}$ was observed for UA ultrasonication pretreatment with acid, while the lowest hydrogen yield of 46 ml/g $VS_{initial}$ was observed for only base pretreatment. Also Nguyen *et al.* (54) investigated the hyperthermophilic H_2 fermentation using rice straw pretreated through a combination of 10% ammonia and 1.0% diluted sulfuric acid and found that the combined pretreatment method significantly increased the digestibility of rice straw and H_2 yield.

Except the inhibitors of hydrolysates, the accumulation of reducing sugar can also inhibit the hydrolysis, finally, causing a dropping H_2 fermentation. To eliminate this inhibition, process configurations such as simultaneous saccharification and fermentation (SSF), simultaneous saccharification and co-fermentation (SSCF) and consolidated biomass processing (CBP) had been conducted (6). Currently, SSF and SSCF have been recognized as feasible options for H_2 production from lignocellulosic materials. In these methods, the hydrolyzed products are immediately consumed by fermentative bacteria for H_2 production, thus, the concentration of monosaccharide remain a very low level in the medium and inhibition of reducing sugar to hydrolysis is eliminated, consequently, enhancing hydrolysis efficiency and H_2 yield. This simultaneous and complete substrate utilization from complex lignocellulosic biomass will bring an energy-efficient process and is a promising method in industrial scale production. However, hydrolysis of cellulose and hydrogen fermentation of the hydrolysates by anaerobic bacteria can't easily proceed in one bioreactor because the optimal conditions of the hydrolysis are significantly different from that of the metabolism of bacterial cells.

Currently, a novel microbiological electronic cells (MEC) method based on the simultaneous enzymolysis and fermentation of cellulosic raw materials has been proposed to produce H_2 under a low external voltage input. In a MEC with two chambers, where both the anode and cathode are operated anaerobically and separated by a proton (cation) exchange membrane, anaerobic bacteria attached to the anode consume substrate using the electrode as an electron acceptor and produce CO_2, protons and some organic acids, thereby generating a low electrical potential, while the protons transferred into cathode chamber accept the electrons from cathode to generate H_2 (Fig. 3). It can be seen that the H_2 purity of the evolved gas can be significantly elevated due to H_2 evolution in cathode and CO_2 generation in anode (55). Thygesen *et al.* (56) found that during the MEC process, 61% of the chemical oxygen demand was removed with the average H_2 production rate of 0.61 m^3/m^3 MEC/day and the total energy production yield of 78% when considering the energy content in the consumed compound.

CONCLUSIONS

Biohydrogen production from lignocellulosic materials has been established as a prospective alternative and integral component of green sustainable energy. Prior to hydrolysis, the lignocellulose must be pretreated by physical, chemical or thermol methods to destroy the incorporated heterogeneous and crystalline structure. The hydrolysates always vary with the used materials and pretreatment methods. Meanwhile, further conversion of hydrolysates to inhibitive compounds always occurs in severe conditions and affects the subsequent H_2 production by fermentation. The appropriate hydrolysate compositions can dramatically improve the biological activities and thus bio-H_2 production performances. Currently, some innovative methods such as simultaneous saccharafication and

fermentation, microbiological electronic cell have been adopted to enhance H_2 production performance.

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