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Research and development perspectives of lignocellulose-based biohydrogen production

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ABSTRACT

Hydrogen production from lignocellulosic biomass (LCB) using dark fermentation is an interesting research niche being developed over the last decade. This review analyses the relevant studies which focused on biohydrogen production from LCB using dark fermentation techniques in terms of substrate characterization, bottlenecks associated with the pretreatment and its subsequent utilization, possible remedies for the scale-up of the most adapted processes and finally the prospects and suggestions which may be envisaged. Studies dealing primarily with the utilization of raw and pretreated LCB have been assessed in terms of biohydrogen production performance for production rate and yield. Energy production analysis and prospecting of suitable cellulosic biomass and efficient cellulolytic microbes have been elucidated towards better cellulose hydrolysis and efficient conversion of LCB to H_2 in addition to process economics.

1. Introduction

Biohydrogen production via dark fermentation is a propitious technique, yet to become an innovative sustainable technology, which may be harnessed to address the energy-related issues of the current and future needs. The unique characteristics of biohydrogen namely its high energy content, zero greenhouse gas emissions during its combustion and also the production possibilities from various organic feed stocks which are abundant in the world would make it an environmentally sustainable fuel (Kumar et al., 2015). Hallenbeck (2009) has mentioned that research on dark fermentative biohydrogen production has been constantly gaining an increasing quantum of interest and a number of biomass-based substrates have been tested using different microbes. Besides, hydrogen production by dark fermentation under the continuous mode and mesophilic condition proceeds essentially with no methane generation in the pH range of 4.5–6.7 and hydraulic retention time (HRT) range of few hours to three days depending on the mix of substrates (Hawkes et al., 2007).

The formation and the effects of inhibitors on biohydrogen producing microbial community have already been detailed in previous reviews and by Bundhoo and Mohee (2016). Jönsson and Martín (2016) have indeed recently cautioned that pretreatments of lignocellulose materials produce inhibitors which lowers the activity of microbes and other biocatalysts, and that the issues related to such inhibition will tend to become more pronounced as the need to process more bio-based materials will arise with the need to produce more bioenergy in a greener and more efficient manner. Cao et al. (2010) have reported that furfural,

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hydroxymethylfurfural, vanillin and syringaldehyde had impeded the growth of *Thermoanaerobacterium thermosaccharolyticum* W16 and negatively affected the biohydrogen generation patterns when corn stover acid hydrolysate was used as substrate. Quemeneur et al. (2012) have also assessed the effects of furan derivatives and phenolic compounds as pretreatment co-products on the overall production kinetics of hydrogen from xylose, and observed that the latter inhibitory species severely hampered the generation of biohydrogen when *Clostridium beijerinkii* was used. Siqueira and Reginatto (2015) have also concluded that 4-hydroxybenzoic acid had the most significant extent of inhibition to the maximum rate of biohydrogen production from glucose, followed by 5-hydroxymethylfurfural and furfural which equally had a (yet lesser) negative impact on the hydrogen production kinetics.

The detoxification methods for the pre-hydrolysates obtained via pretreatment are also explained elsewhere (Kumar et al., 2015). However, these methods could add extra costs to the overall process, thus justifying the need to elaborate an integrated process which avoids these steps. Alternative techniques such as simultaneous absorption of inhibitors during H_2 production by fermentation and the development/isolation of H_2 producers which could tolerate the high levels of inhibitors should be researched more for making biohydrogen from LCB a possible workable solution, at least at a pilot-scale for relatively small units of energy consumption.

1.1. Hydrolysis for cellulose release

Recently, LCB has been put to perspective for biofuel production, especially for biohydrogen generation due to its remarkably high carbohydrate content in the form of hemicellulose and cellulose (Kumar et al., 2008, 2009; Mosier et al., 2005; Schwarz, 2001). The complexity of lignocellulosic biomass originates from the polymerized-form sugars which are covered by a protective lignin layer, which is not easily broken down to eventually set free the carbohydrates (in the form of cellulose and hemicellulose). Once cellulose and hemicellulose polymers are free from the lignin, various methods may be adopted for their hydrolysis using favourable enzymatic processes relying on hemicellulases and cellulases action (Kumar et al., 2009; Mora-Pale et al., 2011; Wilson, 2011; Yamada et al., 2013).

Enzymatic hydrolysis of cellulosic polymer chain is a tedious task and only a smaller fraction of the microbes with cellulolytic capability can fulfill it. The main step of cellulose depolymerization is to cleave the β-1,4 linkage existing between two neighbouring glucose molecules repeatedly all the way along the cellulose chain. In other words, the specialty of cellulase among other enzymes is its degradation potential for the insoluble glucose-polymer, cellulose (Wilson, 2011). Cellulases are classified as endo- and exo-cellulases and act differently. The endo-cellulase binds at random places of the cellulose polymer chain and cuts the molecule into smaller oligomeric fragments, to which exo-cellulase attach and split cellobiose (dimeric) molecules (Rouvinen et al., 1990; Spezio et al., 1993). Finally, cellobiohydrolase is needed to decompose the dimeric sugar obtained into monomeric and consumable glucose. Attributed to their observable synergistic effect, the application of a mixture of cellulases has been described to be more profitable instead of a single class of cellulase enzyme (Ding et al., 2008; Irwin et al., 1993; Wilson, 2008).

Until recently, it was believed that only microorganisms produce cellulases, however, it has been understood that some of the insects, molluscs, nematodes and protozoa are also able to do it. Besides, the enzymes (cellulases, xylanases and hemi-cellulases) they produce also aid in the hydrolysis process. Earlier reports have discussed this positive development in more detail (Wilson, 2011; Yamada et al., 2013). Recently, in an attempt to use green liquids, Pang et al. (2016) have used an oxidative ionic liquid to overcome the refractory characteristics of lignocellulose in an enzymatic process and reported that their pretreatment method had promisingly improved the extraction of lignin.

1.2. LCB pretreatment and hydrogen yield

Karimi and Taherzadeh (2016) have recently comprehensively discussed the use of more accurate techniques to assess the effects and efficiency of different physical, thermal, chemical and biological pretreatments of lignocellulosic materials based on parameters related to imaging, composition of substrates, crystallinity of structures, degree of polymerization of simple molecules, enzymes adsorption and their desorption patterns, and accessibility to the species which are more susceptible to microbial action. Sindhu et al. (2016) have recently reported that one possible limitation to pretreatment is the relatively long duration for incubation which eventually leads to effective delignification of large lignin-based polymers, and they indicated that the choice and action of a properly selected and suitable microbial culture may work out as a possible solution in such a scenario. Kumar et al. (2016) have recently argued that a more effective production of biohydrogen by dark fermentation may be achieved with bioaugmentation techniques which target an enhancement in the quality and action of microbial cultures.

The detailed pretreatment methods and the LCB used in hydrogen production by fermentation are provided in Table 1. Notably, mainly raw LCB have provided the lower yields, and the lower production rates are seemingly due to their low accessibility by microbial populations. Sinha and Pandey (2011) have reviewed comprehensively many of the aspects which are limiting the enhancement of the fermentative production of hydrogen. Amongst the several interesting points they discussed, the possible means proposed to enhance the overall yield of hydrogen production were through the removal of dissolved gas and by gas sparging; by using hybrid systems and by entrapment techniques in reverse micelles. Sinha and Pandey (2011) explained that reverse micelles are self-arranging nanostructures which accommodate entrapped enzymes having higher stability and better biokinetics. Cao et al. (Cao et al., 2009) investigated the biohydrogen production from sulphuric acid-pretreated corn stover by using *Thermoanaerobacterium thermosaccharolyticum* W16 and studied the effect of acid concentration and reaction time on hydrogen yield based on a 2^2 experimental design method. Their results of thermophilic fermentation showed optimal parameters as 1.69 wt% acid concentration and 117 min pretreatment time which resulted in 2.24 mol H_2/mol sugar hydrogen yield. On the other part, biohydrogen production from corn stover by *T. thermosaccharolyticum* W16 integrated with NaOH pretreatment and cellulase enzymolysis was reported by Ren et al. (Ren et al., 2010), wherein the outcomes of the hydrolysate-based fermentation were as follows: 108.5 mmol/L H_2 concentration and 11.2 mmol/L h hydrogen production rate. The latter data recorded were comparable with the results of experiments carried out with model sugar substrate solutions by Liu and Cheng (Liu and Cheng, 2010). The latter had used mixed microbial culture in thermophilic hydrogen fermentation from corn stover and supported the sulphuric acid pretreatment with microwave irradiation. This combined method made it possible to reach 1.53 mol H_2/mol glucose maximal yield by using 0.3 N H_2SO_4 and 45 min contact time, which is significantly higher than the hydrogen yields of fermentative processes from untreated or acid-pretreated corn stover.

Puhulwella et al. (2014) have investigated biohydrogen generation from glucose using *Clostridium butyricum* CWBI1009 under mesophilic conditions in a trickling bed sequenced-batch

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