International Journal of Medicine and Life Sciences

Volume 3, Issue 2, December, 2018

http://www.hknccp.org

Publisher: HongKong New Century Cultural Publishing House
Address: Unit A1, 7/F, Cheuk Nang Plaza, 250 Hennessy Road, Wanchai, Hong Kong

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International Journal of Medicine and Life Sciences
ISSN: 2518-0223, Volume 3, Issue 2, December, 2018

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Novel Fungal Group Pretreatment of Waste oat Straw to Enhance Economic And Efficient Biohydrogen Production

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Abstract: Application a bio-pretreatment to lignocellulosic biohydrogen production was explored with afungal group.Aspergillus nigerand Phanerochaete. chrysosponiumwere used for oat straw pretreatment both individually and in combination. The effectiveness of bio-pretreatments was assessed on the basis of lignin removal, microscopic changes of cell-wall structure and the impact on hydrogen yield. The fungal group of effectively degraded the straw ligninfrom 22.6% to 11.9% (wt% of drywt.) in 7 days.SEM analysisshowed that the cell-wall structure of oat straw was decomposed after the pretreatment of the fungal group. The lignin removal is positively related to the hydrogen yield increasing. Compared to the untreated group, hydrogen produced from the straw pretreated by fungal group increased 165% after 6 days fermentation, which is more than that from the straw pretreated by any one fungi speciesalone (94% for A. niger and P. chrysosponium for 106%).

Keywords: Bio-hydrogen; Fungal group; Pretreatment; Straw; Lignin degradation

1. Introduction

Reducing the reliance on fossil fuels, by converting abundant lignocellulosic biomass to biofuels presents a viable option (Chang et al., 2011).Straw is one of the major lignocellulosic wastes produced during agricultural cultivation of cereals in temperate climates. Its hemicellulose typically contains high fractions of pentose and has a dried-lignin content of 16–25% (Rio et al., 2012). Oat straw is one of the major crops grown in Russia, North America and Europe with around 66.3% carbon hydrates according to the Report of USDA Database (USDA, 2013) Converting agricultural waste such as oat straw to fuel achieve both waste to resources and potentially reduce the fossil fuel effect on climate change.

As hydrogen is a clean energy that could be produced from waste materials, such as organic waste crop straw (Pakarinen et al., 2008), it is believed that hydrogen will replace fossil fuels as the next generation of energy (Verhelst, 2014).Considerable research in recent years has been focused on the conversion of biomass reproducible resources to hydrogen (Pakarinen et al., 2008; Kim et al., 2012; Wu et al., 2013). Biological hydrogen production is now regarded as one of the most promising technologies in the success of fuels evolution (Das, 2009).

Despite previous efforts, the biohydrogen production from lignocellulosic wastes is still challenged by its low conversion efficiency.The main reason is that, the cellulose fraction of strawis recalcitrant to enzymatic breakdown due to the complex structure of cellulose and hemicellulose rigidly covered by lignin resulting inthe stability of its biomass(Balat,2011;Monlau et al., 2013). Therefore, pretreatment of the straw isnecessary to soften lignin component, so that it can be made accessible to cellulose and hemicellulose for microbes used in hydrogen production.A variety ofpretreatment techniques have been developedto improve decomposition of cellulosicfibers including physical (Bak et al., 2009; Wu et al., 2013), chemical (Ballesteros et al., 2008; Zhao et al., 2009), and physic-chemical (Chen et al., 2008). However, chemical methods, such as using acid or alkaline, leave strong acidic, basic, or toxic residues in the treated biomass, having significant environmental risks (Wu et al., 2013). Although Physical pretreatment, such as thermo, microwave and freeze, doesn't use harmful chemicals, it needs large amount of energy input.In addition, most of those processes produce inhibitcompounds, such as weak acids, furan derivatives, and phenolic compoundsfor subsequent fermentation to produce hydrogen(Palmqvist and Hahn-Hagerdal, 2000).

Biological pretreatment is an attractive pretreatment method since it does not involve hazardous processed chemicals and metabolite repression problems (Kausar et al., 2010), and has remarkable advantages of simplicity, low cost and low requirements for equipment (Mshandete et al., 2008).Recent researches have found that some fungal groups are able to degrade the cell walls of straw through composting, such as T. viride and A. niger(Kausar et al., 2010, 2011, 2013), andT. virideand Phanerochaete chrysosporium (Lin et al., 2011)used in composting at around 40-50°C. To the author's knowledge, there isno study on fungal group pretreatment of oat straw for hydrogen production that has been reported. In this study, we studied a low-cost and environmentally sound alternative by using fungal groupto enhance the efficiency of lignocellulosic biohydrogen production. Additionally, the fungal biopretreatment is carried out in a low energy consuming way at atmospheric pressure with less heat use.Two fungal species (A. nigerand P. chrysosporium) were selected as a group working to pretreat the oat straw for facilitating subsequent hydrogen fermentation. The effectiveness of the pretreatment was evaluatedaccording to the lignin removal, SEM analysis and digestibility enhancement for biohydrogen production

2. Materials and Methods

2.1. Rawmaterial

The oat straw obtained from a farm in Richmond, a suburb of Vancouver, Canada. The straw waste was cut to ~1-2 cm long, then milled and sieved through a 2.0 mm screen, and dried at 70 ± 1°C for 4 hours. The driedstraw was then ground into powder and stored in a sealedplastic bag at room temperature.

Two fungal speciesfor the pretreatment, A. niger and P. chrysosporium, were isolated from culture collection by College of Architecture & Environment in Sichuan University, Chengdu, China.Each single colony of the two species and their combination. were enriched respectively at 28± 1°C by shaking at 150 rpm for 3 days in the Mandels medium [what is this?], modified as proposed by Mandels and Weber (1969): 22 g ammonium tartrate; 20 g glucose; 20 g KH2PO4; 8.7 g MgSO4; 1.0 g CaCl2; 0.6 g NaCl; 0.35 g MnSO4; 60 mg FeSO4; 110 mg CoCl2; 60 mg ZnSO4; 95 mg CuSO4; 6 mg H3BO3; 6 mg Na2MoO4 and 100 mg VB1 in 1L deionized water.

Cow manure was used as digesting microflora for hydrogenproduction, was obtained from a farm in a suburb of Vancouver, Canada. Cow manure washeat-treated by boiling for 30 min to inactivate H2-consuming bacteria and to enrich spore-forming H2 producers (Chang et al., 2011). The nutrient stock solution for hydrogen fermentation contained 4 g yeast extract, 12.4 g of KH2PO4; 0.1 g of MgSO4.7H2O; 0.01 g of NaCl; 0.01 g of Na2MoO4.2H2O; 0.01 g of CaCl2.2H2O; 0.015 g of MnSO4.7H2O and 0.0278 g of FeCl2 per liter, which was modified from Lay et al. (1999).

Thephysical and chemical characteristics of raw oat straw and cow dung are summarized in supplemental information, Table S1 and S2.

2.2. Fungal pretreatment of oat straw

For bio-pretreatment, a fungal group of A. nigerand P. chrysosporiumwas chosen as microbial inoculum, and each member was used for comparison. The substrate was inoculated with 1 ml of inoculum at a concentration of 106 CFU in 250 ml Erlenmeyer flasks containing 100 ml ofMandels medium,as described above in section 2.1 except glucose replaced by 6 g of dried oat straw. The culture was incubated at 28 ±1°C for 14 days with shaking at 150 rpm. The straw was then washed with deionized water three times to remove any by-products and dried at 105 ± 1°C for 24 h for further analysis and nexthydrogen fermentation.

2.3. Degrading effects of pretreatment

The residue of the straw was collected to determine the cellulose, hemicellulose and lignin contents according to Goering and Van (1970).The reducing sugar content of the pretreated samplesproduced for each incubation time was analyzed by the dinitrosalicylicacid (DNS) method (Miller, 1959).

Scanning electron microscopy (SEM) investigations of the effects of fungalpretreatment on straw cell wall disruption, composition, ultrastructure and surface properties were carried out in order to better understand the increased susceptibility to digestion in hydrogen fermentation (Kristensen et al., 2008). SEM was carried out in a JEOL microscope model JSM-5900. Prior to analysis the samples were coated with gold to a thickness of ~7 nm using a Polaron SC7620 sputter coater. Microscopy was performed at 10 and 12 kV acceleration voltage and with magnification ranging from 50 to 1,000.

2.4. Anaerobic fermentation of hydrogen production

The anaerobic experiments were performed with 150 ml serum vials as batch reactors containing the mixture of 6 g of the pretreated oat straw, 6 g cow dung and 60 ml of nutrient stock solution. Untreated oat straw was used as a control. These vials were infused with nitrogen to remove oxygen from the headspace of the reactors so as to keep the anaerobic condition. The bottles were incubated in an orbital shaker at 65°C, with a rotation speed of 90 rpm to provide better contact among substrates (Sheffield J.W. and Sheffield C., 2007). The volume of biogas was determined using glass syringes of 5 to 50 ml.

The gas composition (H2, CH4 and CO2) was analyzed with a gas chromatography (GC, Agilent 4890D)

equipped with a thermal conductivity detector (TCD) and a 6-foot stainless column packed with Porapak Q (80/100 mesh). The operating temperatures of the injection port, oven and detector were 100°C, 80°C and 150°C, respectively (William et al., 2015). Nitrogen was the carrier gas at a flow rate of 20 ml/min.

2.5. Data analysis

The hydrogen yield (ml/g straw) was obtained by dividing the cumulative hydrogen production (ml) by the dry weight of straw used for fermentation. Analysis of variance (ANOVA) was used to estimate the statistical parameters.

3. Results and Discussion

3.1. Effects of fungi group on lignin degradation

Results of the effects of twoindividual fungi species and their combination on lignin degradation are plotted inFig. 1. As shown in Fig. 1, the group of A. nigerand P. chrysosponium,showed better degradation effects onoat strawlignin than any one of them alone, and the delignification increased with the time increasing (Fig. 1). After fungal group digestedfor 3 days, the lignincontent decreased to 17.4% from 22.6% (wt% of drywt.). As the pretreatment timeextended to 5 days, the lignin content decreased to 14.4%;when the period increased to 7 days, the lignincontent further decreased to 11.9% with total lignin removal of 47.35% (Fig. 1).

As mentioned above,lignin encloses cellulose and hemicellulose molecules, makingthem difficult to reach, therefore, lignin content and distributionconstitute the most recognized factor responsible for recalcitranceof lignocellulosic materials to enzymatic or microbial degradation (Wu et al., 2013). However, P. chrysosponium was proven highly reactive for lignindegradation in early and recent years (Michael and Margaret, 1993; Zeng et al., 2014). A. niger showed a significant celluloytic power to rice straw and obtained decrease in the straw cellulose and hemicellulose contents, although lignin is recalcitrantto many other degradation methods (Kausar et al., 2010). In the present study, the specific fungal group displayed synergistic effects, which degradedthe oat straw lignin more effectively than using only one fungi species, indicating that fungal group fermentation could be potentiallya good pretreatment option to enhance biohydrogen production fromlignocellulosic biomass.

As can be seen in Fig. 1, longer extending the processing time from 7 days to 14 days did not significantlyincrease lignin degradation. Therefore, A. nigerand P. chrysosponium group with 7 daysprocessing time was selected as the applied effective bio-pretreatment for the subsequentexperiments.

3.2. Effects on the straw cell-wall matrix

The result of SEM showed that the surface of oat straw particleschanged dramatically with fungi pretreatment. Images of the untreated oat straw particles revealed the elongatednature of the cells in the inner parts of the straw, and displayed the smooth, palisadic surfacesof the epidermal layers of oat straw, whichappeared resistant to fungal enzymaticattack (Figure 2A-C) as thetubing making up the porous elongated particle structureswere relatively intact.

In contrast, images of the fungi treated substrate samples demonstrated that the fungal group pretreatment resulted in analtered surface structure and rupture of the parenchyma cells(Figure 2D-L). The cell structures seemed more loosely connectedand more exposed cellulosechains or cellulose (micro)fibrils after A. nigerand P. chrysosponium group pretreatment (Fig. 2J-L). The epidermal surface layer appeared more torn and roughedges, andtheparticles only had little intact epidermal structures left, although some areas of palisadic surfaces were stillvisible (Fig. 2L).

However, the images of the strawparticles with one fungal strain treatment revealed partialpresence of the initial structures of the oat straw (Fig. 2 D-I). Mainly granularstructures remained, but ordered, physiological structures,presumably rudiments of the epidermis and xylem, couldalso be seen (Figure 6C-F), whilst after pretreatment, the cell structures were more loosely connected (Fig. 6G-I).In general, compared to the fungal group treated sample, the samples treated by either A. nigeror P. chrysosponiumpresented fewer signs of rupture ofthe internal parencyma, phloem and xylem structures, but crevices and holesappeared after those pretreatments.

These SEM images thus clearlyindicated that remarkable changes of the straw structures made by fungal group, whichthus seemed to result in improved substrate–enzyme interactions.

3.3. Effects of fungi pretreatmenton the straw fermentation of biohydrogen production

Fungi can degrade lignin, it is therefore, logical to expectedthat fungi pretreatment would enhance biohydrogen production. To confirm whether the enhancement of lignin degradation does necessarilyincrease biohydrogen yield.It is, therefore, necessary toconduct an anaerobic fermentation experiment to determine the overalleffect of fungal group pretreatment on biohydrogen production fromthe oat straw.

3.3.1. Effects on biohydrogen yield

A batch fermentation experiment was conducted to examine effectof fungi pretreatment on biohydrogen production. The experiment wasperformed for 8 days with samples pretreated byT. viride,P. chrysosponiumand their group, as wellas the untreated one (the control). Both untreated and bio pretreatedsamples produced hydrogen

(Fig. 3), no methane was detected,indicating the absence of methanogens. As shown in Fig. 3, fungal group pretreatment significantly increased biohydrogen yield more than the single strains and the control.After 6 days of fermentation, the sample pretreated by fungal group produced 82 ml/g straw hydrogen while A. nigertreated, P. chrysosponiumtreated anduntreated samples produced 60, 64 and31ml/g straw, respectively.Compared to the untreated one, hydrogen produced by the fungal group treated straw increased 165%, while the A. nigerand P. chrysosponium treated samples increased 94%and 106% respectively.This hydrogen yield is similar to previous studies on hydrogen production by the pretreatment options of physical, chemical and thermal (Chang et al., 2011;Kim et al., 2012; Wu et al., 2013). Furthermore, the lag stage of the control was much longer than fungi pretreated samples. This result suggested that the aerobic digestion may have broken lignin barriers in cell walls for improved substrate conversion and shortened the start time of anaerobic fermentation.

Fig. 4saw the changes in accumulative hydrogen and carbon dioxide yield during the conversion of the fungi pre-digested oat straw wastes to biohydrogen by cow dung compost. The resultindicated that regardless of pretreatment or not, all groups produced similar ratio of H2 to CO2 (4.9–5.3).

As possible inhibitors was removed mostly by washing the substrate before hydrogen fermentation, there was few inhibitory effect was observed with the samples pretreated by fungal group. Currently most existing pretreatment processesform more or less undesirable byproductswhich are inhibitors tothe fermentation processes (Palmqvist and Hahn-Hagerdal,2000). Quéméneuret al. (2012) concluded that all the putative inhibitory compounds place a significant negative impact on H2 productionperformance. However, results of the present fermentation experiment unequivocallydemonstrate that under our experimental conditions the fungal group pretreatmenthad an overall effect on biohydrogen production enhancement.

3.3.2. Straw degradation versus hydrogen production

It is reasonable that when lignin is removed more, the reducing sugar is more. Fig. 5 presented the relationship between sugar yield and the lignin removal in the samples pretreated by thegroup of A. nigerand P. chrysosponium. It clearly proved that the increasing pattern of reducing sugar was similar to the change of the lignin removal. These results are in agreement with those reported(García-Cubero et al., 2009; Schultz-Jensen et al., 2011).

It is assumed that fungi digestion of oat straw decreased insoluble lignin content, increasedconsiderably digestibilityin hydrogen production stage by increasing the accessibility in comparison with the non-pretreated raw materials (Singh et al., 2011). Lignin degradation versus hydrogen production was plotted inFig. 6, which showed that hydrogenproduction is positively related to the degradation of lignin in straw. To achieve an efficient biohydrogen production, apretreatment targeting at lignin removal is usually adopted (Renet al., 2009; Chang et al., 2011; Quéméneur et al., 2012) so that cellulose and hemicellulose can be released, exposedto microbes, and subject to enzymatic attack. Results of this study presentedhere (Fig. 6) clearly demonstrated that under the givenexperimental conditions fungal group reduced lignin contents much more, andtherebyenhanced biohydrogen production more efficiently.

4. Conclusions

Fungal group pretreatment effectively degraded the oat straw lignin (47.35% in 7 days) leading to majordecomposition cell-wall structure and thereby increased biohydrogen yield. The hydrogen produced from the fungal group pretreated straw increased 165% 6 days later, which was more than that produced from either one fungi specie of A. nigeror P. chrysosponium pretreated straw (94% and 106%, respectively). No inhibitory effect on hydrogen production wasobserved with all samples.Overall, this fungal group gave thehigherhydrogen yield, indicatingthat productionof biohydrogen from oat strawcan be significantly enhanced by A. nigerand P. chrysosponium group pretreatment.

5. Acknowledgements

This research, as a collaborative project between the University of British Columbia (UBC) andSichuan University, was funded by Science & Technology Department of Sichuan Province (No. 2014FZ0027) and the Natural Sciences and Engineering Research Council of Canada.

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