Hydrogen Production Potential of Wheat Straw by Enzymatic Saccharification Andfermentation

Sandeep Kumar*[1], Shiv Prasad[1] & Navindu Gupta[1]

[1] *Centre for Environment Science and Climate Resilient Agriculture,*
Indian Agricultural Research Institute, New Delhi – 110012, India

---

Abstract: Biological hydrogen production (H_2), especially microbial biohydrogen production provides a feasible means for the sustainable supply of H_2 with low pollution and high efficiency, thereby being considered as a promising way of producing hydrogen, and it had got special attention in the last decade. Wheat straw is an abundant agricultural residue, and composed of heterogeneous complex of carbohydrate polymers, especially cellulose and hemicelluloses, which can be readily hydrolyzed into fermentable sugars by pretreatment and enzymatic saccharification.

Hydrogen producing microorganisms can convert these hydrolyzed sugars into hydrogen. In the present investigation, pretreated straw of the wheat varieties C 306, K68, HD 2967 and WR 544 were taken for enzymatic saccharification and hydrogen production. The samples of microwave assisted chemical pretreated straw of the wheat varieties were processed for enzymatic saccharification with cellulase and β-glycosidase enzymes. The total fermentable sugars from pretreated and saccharified straw were ranged from 46.38 to 67.49%. The saccharified hydrolysates were fermented into hydrogen by using microbial strains of Bacillus coagulans NCIM 2323 and Enterobacter aerogens NCIM5139. The Hydrogen yield in terms of mol/mol glucose ranged from 0.23 to 1.40. A maximum hydrogen yield up to 205 mL/g of sugar (equivalent to wheat straw) was obtained by Enterobacter aerogens NCIM 5139. Maximum hydrogen production potential was observed to be from the wheat varieties HD 2967, followed by WR 544. Thus wheat straw can be a potential feedstock for hydrogen production.

Keywords: Wheat straw, enzymatic saccarification, sugar, Bacillus and Enterobacter aerogens strains, hydrogen production.

1. INTRODUCTION

Today, global energies are mostly produced from fossil fuels. This increasing demand of energy will lead to the foreseeable depletion of limited fossil energy resources (Das *et al.,* 2001, Levin *et al.,* 2004). Increased production of H_2 by current technologies will consume greater amounts of natural gas, which in turn will generate greater greenhouse gas (GHG) emissions (Manish and Banerjee, 2008). So that by replacing the use of non-renewable fossil fuel feedstock, the increase in

---

GHG emissions can be reduced through CO_2 sequestration. Production of H_2 from renewable sources derived from agricultural residues or other waste streams offers the possibility to contribute to the production capacity with lower or no net greenhouse gas emissions, increasing the flexibility and improving the economics of distributed and semi-centralized reforming (Demirbas, 2001).Wheat straw is one of the most abundant agricultural residues in some part of India. Heterogeneous complex of carbohydrate polymers, especially cellulose and hemicelluloses from wheat straw, can be readily hydrolyzed into fermentable sugars by pretreatment and enzymatic saccharification and subsequently hydrogen production by microbes (Jorgensen *et al*., 2007).

2. MATERIALS AND METHODS

2.1. Substrate for saccharification

The wheat straw of the varieties C 306, K 68, HD 2967 and WR 544 was pretreated with microwave assisted acid at 140˚C, 180˚C and 200˚C for 10 min, samples were washed and neutralized, before the saccharification (Brown and Torget, 1996).Sample equal to the equivalent of 0.1g of cellulose was taken in 250 mL conical flasks, and to each flask 5 mL of 0.05 M sodium citrate buffer (pH 4.8), cellulase enzyme to equal approximately 60 FPU/g cellulose, β-glucosidase to equal 64 pNPGU/g cellulose, 20mg/mL sodium azide as antibiotic to prevent growth of organisms during the process and then made up to 10 mL with distilled water.All the experiments were done in triplicates. Total soluble sugar was analysis by anthrone method (Thimmaiah, 2006).

2.2. Organisms growth and experimental setup

The hydrogen producing strains (*Bacillus coagulans NCIM* 2323 and *Enterobacter aerogens NCIM* 5139) were sub-cultured with Nutrient Agar Media (g/L): Beef extract, 10.0; NaCl, 5.0; Peptone, 10.0; Agar, 20.0; Distilled water, 1.0 L; pH adjusted to7.0-7.5. The experimental setup was carried out in standard 1 liter conical flask (Borosil) with wheat hydrolysate (250ml), sealed with air tight rubber crock and packed with para film for the anaerobic condition as well as to stop the leakage of gas produced by microbes.The quantity of gas produced by each strain was evaluated volumetrically in mL described by Papgeorgiou*et al*., 2007.

The hydrogen concentration was analyzed by a gas chromatograph ((Shimadzu GC-14B, Japan), using TCD, and argon as carrier gas at flow rate of 10 mL/min, equipped with Porapak Q stainless steel column (180 cm long, 3 mm outer diameter). The injector, detector and oven temperatures were 120, 150 and 50°C, respectively.Estimation of Hydrogen yield (Equivalent to 1 mol of H_2) was calculated as per following procedures:1 mol of glucose is equivalent to = 180 g, 1 mol of H_2 is equivalent to = 24800 ml (24.8 L) at STP, Hence: 180 g of glucose gives 24800 ml $H_{2,}$ However, 1 g glucose gives 24800/180 = 137.7 ml (Equivalent to 1 mol of H_2).

3. RESULTS AND DISCUSSION

3.1. Saccharification of pretreated wheat straw for sugar recovery

Microwave assisted acid pre-treated wheat straw was subjected to saccharification for the recovery of sugar (fermentable sugar). After saccharification, at 140˚C, 180˚C and 200˚Cfor varieties HD 2967, WR 544, K 68 and C 306, the sugar content recovered ranged from 25.6 to 56.8%(Fig. 1). Combination of microwave assisted acid treatment at lower temperature has been viewed as an alternative for the high temperature (>160°C) treatment of wheat straw, which leads to loss of sugars. The maximum total soluble sugars yield were obtained from wheat straw at 160°C for 10 min (Fig. 1).

3.2. Total soluble sugars from pretreatment and enzymatic saccharification

The result observed for total sugar recovery after microwave assisted acid pretreatment and saccharification of the different wheat varieties is given in Fig. 2. It was found maximum in HD 2967 (67.49%) followed by WR 544 (56.22%), C 306 (48.06 %), and it was observed to be minimum in K 68 (46.38%). Microwave assisted acid pretreated saccharified and gave higher sugar recovery than control (Fig. 2). The result is consistent with the findings of many researchers Singh *et al*. (2010) and Zhu *et al.* (2005) who also reported that microwave assisted chemical treatment is the best pretreatment for enhanced biomass saccharification and fermentable sugar recovery.

3.3. Hydrogen production potential of wheat varieties straw

The sugar obtained after pretreatment and saccharification was subjected to hydrogen fermentation. The volumetric hydrogen yield from wheat varieties were ranged from 62 to 205 mL. A maximum hydrogen yield up to 205 mL/g of sugar (equivalent to wheat straw) was obtained by *Enterobacter aerogens NCIM 5139*(Fig. 3). The hydrogen production potential ranged from 0.23 mol/mol glucose (1^{st} day) to 1.4 mol/mol glucose (7^{th}day). Similar results for H_2 yields mol/mol glucose were also reported by Kraemer *et al.,* 2006; Wang *et al.,* 2009 and Lee, 2010.Maximum hydrogen production potential was observed to be from the wheat varieties HD 2967(1.4 mol/mol glucose), followed by WR 544. It was observed to be lowest in K 68. Among the wheat varieties, HD 2967 was best suitable for hydrogen production**.**

4. CONCLUSION

Biological hydrogen production (H_2) provides a feasible means for the sustainable supply of H_2 with low pollution and high efficiency, thereby being considered as a promising way of producing clean fuel. Wheat straw can be readily hydrolyzed into fermentable sugars by pretreatment, enzymatic saccharification and subsequently hydrogen fermentation. The total fermentable sugar in terms of total soluble sugar ranged from 46.38 to 67.49% from different wheat varieties. The Hydrogen yield in terms of mol/mol glucose ranged from 0.23 to 1.40. A maximum hydrogen yield up to 205 mL/g of sugar (equivalent to wheat straw) was obtained by *Enterobacter aerogens NCIM 5139*. Maximum hydrogen production potential was observed to be from the wheat varieties HD 2967, followed by WR 544. Thus wheat straw can be a potential feedstock for hydrogen production.

5. ACKNOWLEDGEMENT

The authors are thankful to the Post Graduate School and Director, Indian Agricultural Research Institute, New Delhi, India for providing for fellowship towards M.Sc. programme of the first author. We are also grateful to the Centre for Environment Science and climate Resilient Agriculture, Indian Agricultural Research Institute, New Delhi for providing necessary facilities for undertaking this study.

REFERENCE

[1] Brown L, Torget R. NREL Chemical Analysis and Testing Task. Laboratory Analytical Procedure. Enzymatic saccharification of lignocellulosic biomass. LAP- 009, National Renewable Energy Laboratory, Golden, CO.1996.

[2] Das D, Veziroglu T N. *Hydrogen production by biological processes: a survey of Literature*. Int. J. Hydrogen Energy 2001; 26: 13-28.

[3] Demirbas A. *Biomass resource facilities and biomass conversion processing forfuels and chemicals*. Energy Conversion and Management 2002;42: 1357.

[4] Jorgensen H, Kristensen J B, Felby C. *Enzymatic conversion oflignocellulose into fermentable sugars: Challenges and opportunities*. Biofuels,Bioprod. Bioref2007; 1: 119-134.

[5] Kraemer J T, Bagley D M. *Supersaturation of dissolved H2 and CO2 during fermentation hydrogen production with N2 sparging*. BiotechnolLett 2006; 28:1485-91.

[6] Lee D Y. *Continuous H_2 and CH_4 production from high-solid food waste in the two-stage thermophilic fermentation process with the recirculation of digester sludge*. BioresourTechnol2010; 101:42-47.

[7] Levin D B, Pitt L, Love M. *Biohydrogen production: prospects and limitations to practical application*. Int J Hydrogen Energy 2004; 29(2):173–85.

[8] Manish S, Banerjee R. *Comparison of biohydrogen production processes*. Int. JHydrogen Energy2008;33: 279–286.

[9] Papageorgiou N, Ferro Y, Salomon E. *Quantum calculations via densityfunctional theory and photoemission measurements Geometry and electronic structure of lead phthalocyanine*. In Physical Review *B* 2007*;* 68(23).

[10] Singh A, Tuteja S, Singh N, Bishnoi N R. *Enhanced saccharification of rice straw and hull by microwave–alkali pretreatment and lignocellulolytic enzyme production*. BioresourTechnol 2010; 102: 1773–1782.

[11] Thimmaiah. Standard methods of biochemical analysis.2006.kalayani publication.

[12] Wang J, Wan W. *Factors influencing fermentative hydrogen production: a review*. Int J Hydrogen Energy 2009; 34:799-811.

[13] Zhu S, Wu Y, Yu Z, Liao J, Zhang Y. *Pretreatment by microwave/alkali of rice straw and its enzymic hydrolysis*. Process Biochemistry *2005;*40: 3082–3086.

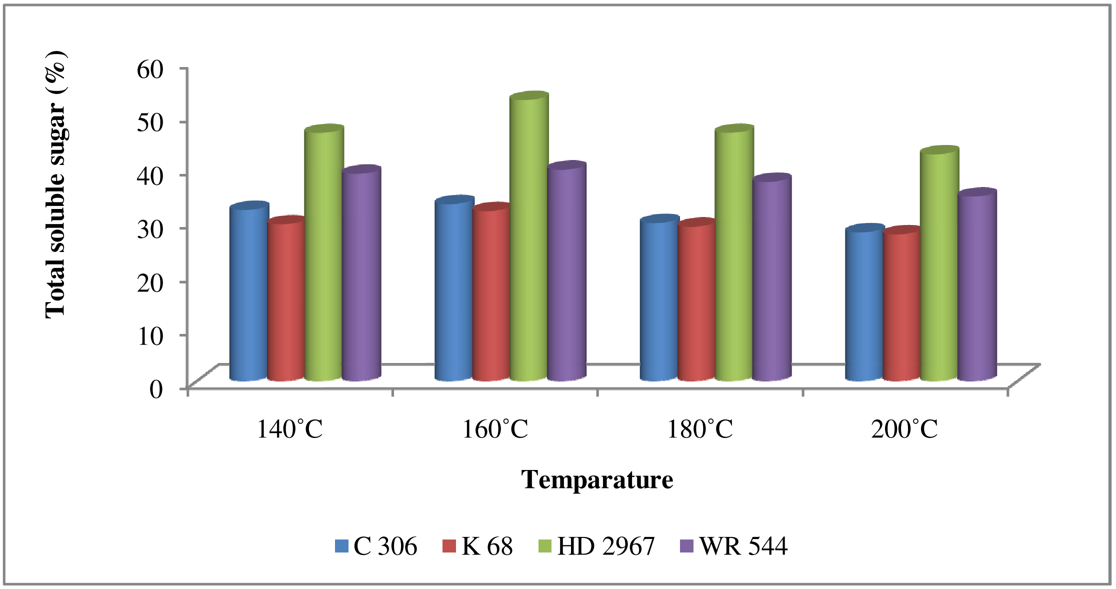

Fig. 1: Total fermentable sugars obtained after saccharification from wheat varieties at different temperature with 10 min.

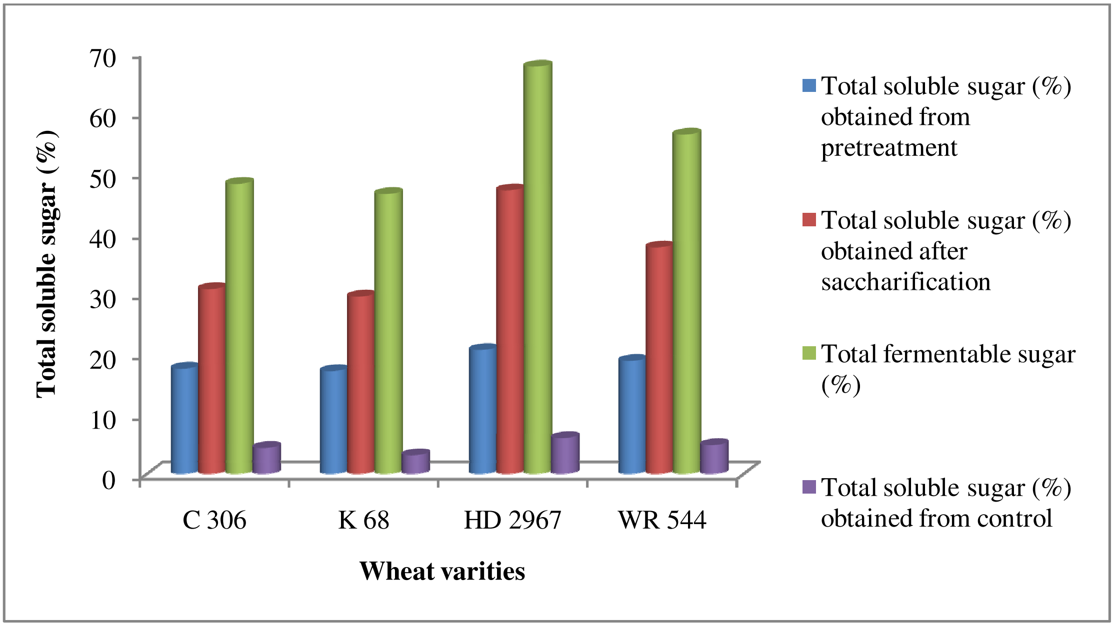

Fig. 2: Total fermentable sugars obtained from pretreatment and after saccharification from wheat varieties.

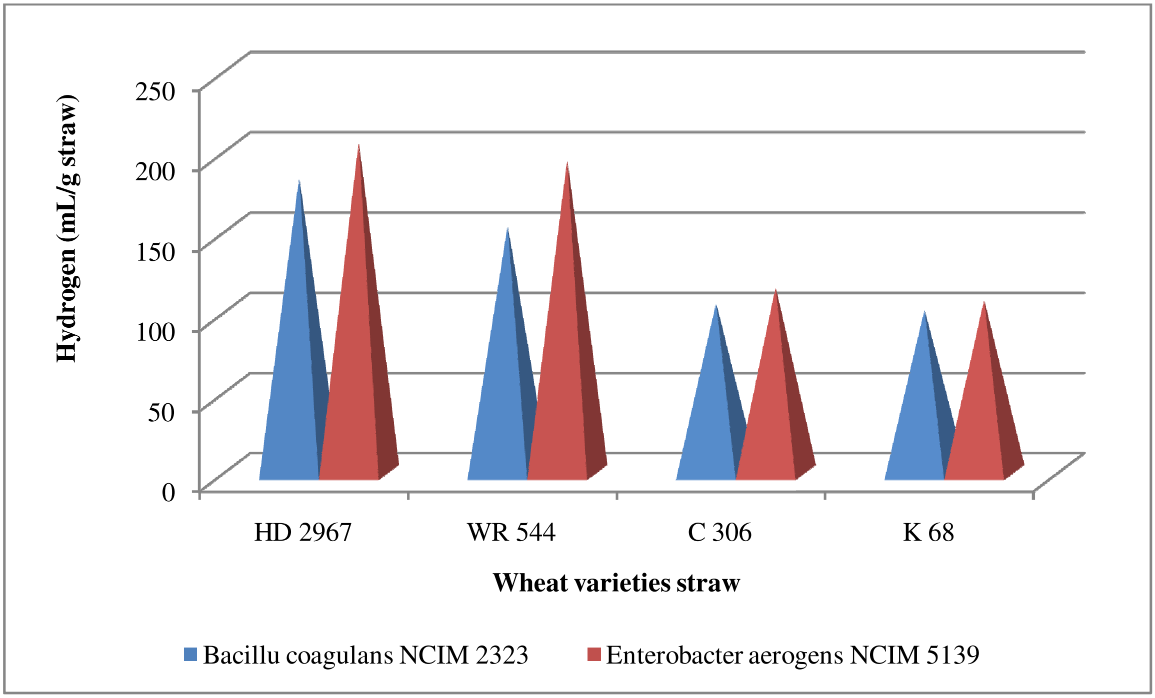

Fig. 3: Potential of different hydrogen producing microbial strains from wheat straw