

Production of Bio-fuel (Bio-Ethanol) from Biomass (Pteris) by Fermentation Process with Yeast

Pradipsaha^{1*}, Md. FakhruAlam¹, Ajit Chandra Baishnab¹, MaksudurRahman Khan^{1,2}, M. A. Islam¹

¹Department of Chemical Engineering and Polymer Science, Shahjalal University of Science and Technology (SUST), Sylhet, Bangladesh

²Faculty of Chemical and Natural Resources Engineering, University Malaysia Pahang, Gambang, Kuantan, Pahang, Malaysia

*Corresponding author: pradip-cep@sust.edu

Received January 04, 2014; Revised January 20, 2014; Accepted January 24, 2014

Abstract Bio-Ethanol is a renewable; eco-friendly energy source can be produced from bio-mass (hemicelluloses). Pteris (fern) is grown very fast and has not major economic importance but it is a reliable source of hemicelluloses can be converted to bio-Ethanol. In the hydrolysis of hemicelluloses the concentration of glucose and the reaction rate was observed with respect to the different parameters, like pH, temperature, substrate diameter, substrate loading. In this study we found optimal parameters, NH₄OH treatment as the best treatment among H₂SO₄, NH₄OH, and NaOH treatment. In addition, pH 7, temperature 35°C, substrate diameter 45µm-63µm, and substrate loading 0.25gm in 100ml working volume was found as optimum operation condition for hydrolysis reaction, which was carried out by *pseudomonas sp.*, isolated from cow dung. In this experiment sugar concentration was measured with UV spectrophotometer using DNS reagent finding the equilibrium time is 72hours for hydrolysis process and the maximum sugar concentration of 1.7625mg/l was achieved. Subsequently we study the fermentation process using yeast to produce Bio-Ethanol from reducing sugar solution obtained from the hydrolysis process on optimum reaction condition and yield the Ethanol concentration 0.333 mg/L, measured with UV spectrophotometer. Furthermore, it was also observed that the activity of yeast was sustain, in the reaction condition (pH = 7, temperature = 25°C), only for 50 hours. In sum, it could be conclude that 0.1332 mg of ethanol can be produce from 1gm of pteris where the conversion of reducing sugar to ethanol is 20% approximately in the optimum reaction condition.

Keywords: Hemicellulose, pteris, hydrolysis, *pseudomonas sp.*, reducing sugar, bio-ethanol, fermentation

Cite This Article: Pradipsaha, Md. FakhruAlam, Ajit Chandra Baishnab, MaksudurRahman Khan, and M. A. Islam, "Production of Bio-fuel (Bio-Ethanol) from Biomass (Pteris) by Fermentation Process with Yeast." *Sustainable Energy*, vol. 2, no. 1 (2014): 25-28. doi: 10.12691/rse-2-1-5.

1. Introduction

With the developing of human life style and civilization, emphasis on energy consumption increases day by day. For this reason different nonrenewable energy sources such as coal, NG, petroleum based fuel etc. are the main targets of many countries. But elevation of CO₂ from this fuel is the most head cable reason considered as a main cause of global warming considered by Omer [1]. Beside this all are the nonrenewable energy source are declared not remain much in reservoir to fulfill the growing demand of the world. For this reason many countries of the world are trying to find out a suitable solution of the growing demand of the fuel with the best consideration of the environment. According to the Suleiman [2] renewable and eco-friendly energy sources with existing fuel energy, tidal energy, hydro energy, geothermal energy, solar energy, wind energy, and bio-ethanol energy from cellulosic materials etc., are the solution to overcome from this upcoming problem. However, bio-ethanol energy from cellulosic materials is ahead from other energy because of availability of the raw materials throughout the year with the as usual property of the other renewable

property such as less emission of greenhouse gas, biodegradable and less toxic etc., simultaneously improve the air quality informed by Charles [3]. DoKyoung et al. [4] found that fern (Pteris) is a celluloses plant which is economically less important and has the three common components (cellulose 30-50%, hemicellulose 20-40% and lignin 15-25%). The cellulose and hemicelluloses part can be hydrolyzed to produce reducing sugar since these are in amorphous state but the Lignin is a complex three-dimensional aromatic polymer hydrophobic in nature investigated by Jenni [5]. The hydrolysis process also depends on the particle size of substrate and loading of substrate. Mohammad. and Keikhosro [6] says that processing of lignocellulosic biomass to ethanol consists of four major unit operations (pretreatment, hydrolysis, fermentation and product separation or purification). Zheng et al [7] and Michelle [8] discussed about different types of pre-treatment techniques available such as chemical pretreatment (acid treatment, ammonia treatment, sulfuric acid treatment alkaline wet oxidation and ozone pretreatment), physical pretreatment (steaming, grinding and milling, blending, thermal, and irradiation), biological pre-treatment and the combine pretreatment. Chemical pretreatment overcome the recalcitrance of lignin present in the structure of hemicelluloses and physical

pretreatment reduce the biomass physical size (increase the surface area). There are two methods generally used in hydrolysis of cellulose acid/base catalyzed hydrolysis and enzymatic hydrolysis. Alexander et al [9] investigated that Enzymatic hydrolysis is better than the acid/base catalyzed hydrolysis. Again there are two available ways of fermentation SSF (Simultaneous Saccharification and Fermentation) and SHF (Separate Hydrolysis and Fermentation). Again among different types of fermentation it is observed by Kim et al [10] that SHF is better than the SSF in many causes. In the present study as chemical pretreatment acid treatment, ammonia treatment, sulfuric acid treatment and Physical pretreatment blending were performed from the available techniques. After pretreatment the hydrolysis performed to produce reducing sugar using cellulase produce from cellulytic bacteria *pseudomonas sp.* and then performing the SHF using *Saccharomyces cerevisiae* (Baker's yeast).

2. Materials and Methods

2.1. Isolation and Identification of Cellulytic bacteria

The cow dung a source of different bacteria was collected from the Toker bazaar, Sylhet and the study was performed in the research laboratory of Shahjalal University of Science and Technology, Sylhet, Bangladesh. The bacterial strain was *pseudomonas sp* identified by the Cowan and Steel's Manual [11] for the Identification of Medical Bacteria.

2.2. Extraction of Enzyme from Bacteria

A loop of bacteria cultured in nutrient Agar media was transfer into the production medium (KH_2PO_4 :1.00gm/L; K_2HPO_4 :1.145gm/L; MgSO_4 : 0.4gm/L; NH_4SO_4 :5.0gm/L; CaCl_2 :0.05gm/L; FeSO_4 :0.00125gm/L; CMC:10gm/L) which was sterilized by autoclaving at 121°C for 15 min. 5 ml of the media was transferred into five screw cap test tubes and kept in a shaker incubator at 37°C for 24 hours at 100rpm, after that each of the 5ml seed culture test tube are poured into other 100ml conical flasks containing 35ml sterilized production media and kept in shaker incubator at 37°C for 24 hours at 100rpm. Subsequently the product was centrifuged for 15 minutes at 8000rpm maintaining 4°C temperatures in refrigerated centrifuge. The supernatant was collected in bayel as enzyme and kept in refrigerator for further use.

2.3. Pre-treatment of Pteris

Pre-treatment is required to achieve best enzyme performance, increasing reaction rate. Chemical pre-treatment were performed using H_2SO_4 acid, NaOH, NH_4OH , and Mechanical pre-treatment is performed by using a blender. The pteris was collected from the university area and washed vigorously with distilled water.

2.3.1. Chemical Pre-treatment

200ml of 1% (v/v) H_2SO_4 , 1% (w/v) NaOH, and 4% (v/v) NH_4OH were taken in separate beakers and 20gm of washed fern was mixed in each of the beaker. The mixture solutions was heated at 80-90°C in an oven for two hours,

then neutralise with distilled water and dried at 105°C temperature for three hours.

2.3.2. Mechanical Pre-treatment

After different chemical pre-treated pteris leaves were dried and blended in a blender and desired particles sizes were separated using the sieve-shaker.

2.4. Experimental Procedure

The hydrolysis and fermentation reaction were performed in the rotary flask shaker (Model: LRD-750) with working volume 100ml in 250ml flasks. Based on the study purposes various amount of substrate with different particle size were taken in the flask. All the composition and parameter assumed same in the total reaction mixture due to continuous rotation. All experiments were run with different predetermined amount of enzyme in the hydrolysis environment with different amount of substrate, different size of particle, different pH, and different temperature to optimize the corresponding parameter. Samples were taken time to time and boiled with DNS solution after centrifuging the sample to destroy the enzymes activity which confirms the reaction ceasing. Then the sample was analysed for glucose in UV spectrophotometer according to the method by Miller [12]. When the hydrolysis reaction reached at its equilibrium then the reaction mixture were separated by filtration method and performing fermentation to produce bio ethanol using *Saccharomyces cerevisiae* (Baker's yeast) and detect the ethanol concentration using $\text{K}_2\text{Cr}_2\text{O}_7$ reagent and UV spectrophotometric method proposed by Adran and Prifysgol [13].

3. Result and Discussion

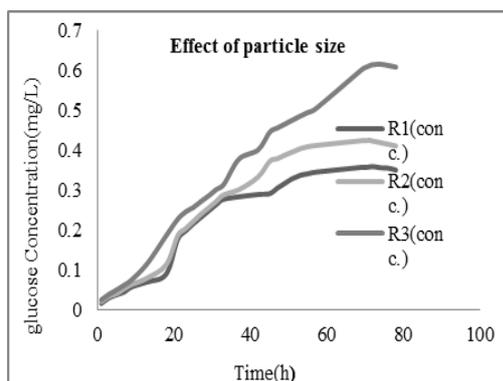
Though the reaction rate depends on different parameters, but in this study we assumed that the rate is affected only by Pre-treatment, Substrate loading, Enzyme loading, Particle size, Temperature, pH. To understand the effect of one parameter other parameters were kept constant and some run with differing the parameter which wanted to investigate.

3.1. Effect of Pre-treatment and Particle Size

Dried equal amount (pteris) substrate were taken by maintaining the other parameters same (particle size 45-63 μm ; pH 7; temperature 298K; [S] 2.5mg/L; [E] 10ml/L) for all three batches. The study shows that NH_4OH treatment has highest amount of reducing sugar concentration among all other treatment presented in Table 1. From the experiment done by Mark and Arthur [14] this consequence may be occurred due to higher removal of lignin, no formation of barrier for the hydrolysis and easily product recovery as ammonia gas. Again in the Figure 1 it is seen that the reducing sugar concentration is increased up to 72 hours from the starting for different particle size (45-63 μm , 63-125 μm , and 125-250 μm). But for the particle size 45-63 μm high amount of reducing sugar is obtained because for that size of particle the surface area of substrate was higher than the others. From this study it can be considered that the optimum size is 45-63 μm and pre-treatment is NH_4OH treatment.

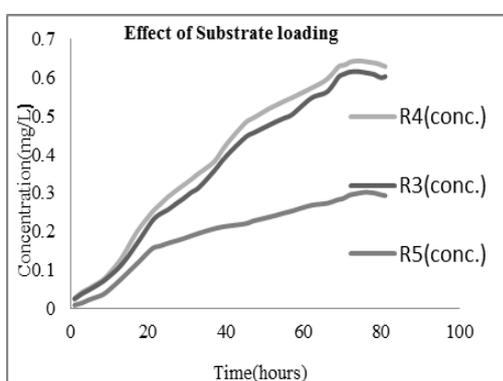
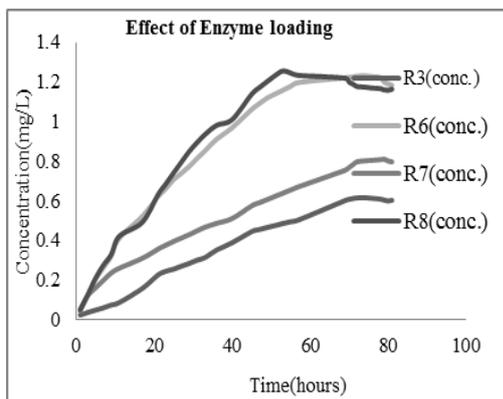
Table 1. Investigation of the effect of pre-treatment

Treatment name	Reducing sugar con.(mg/ml)
NH ₄ OH treatment	0.642
H ₂ SO ₄ treatment	0.604
NaOH treatment	0.612

**Figure 1.** Investigation of the effect of particle size [R1 = 45-63 μm; R2 = 63-125 μm; and R3 = 125-250 μm]

3.2. Effect of Substrate and Enzyme Loading

Different amount of NH₄OH pre-treated fern (pteris) substrate (1.25gm/L, 2.5gm/L, 3.75gm/L) having 45-63 μm particle size and different amount of enzyme (10ml/L; 20ml/L; 30ml/L; 40ml/L) was inserted to investigate the effects. The both study was performed maintaining pH 7; temperature 298K. The study shows that 2.5gm/L is optimum though for 3.75gm/L substrate the glucose concentration is slightly higher than for 2.5gm/L but the reaction rate is comparatively slow due to substrate to enzyme concentration ratio increased investigated by Kristensen [15] shown in Figure 2.

**Figure 2.** Effect of substrate loading. [R3 = 2.5gm/L, R4 = 3.75gm/L, R5 = 1.25gm/L]**Figure 3.** Effect of Enzyme loading. [R3 = 10ml/L R6 = 30ml/L, R7 = 20ml/L, R8 = 40ml/L]

Again Figure 3 shows that the concentration of the reducing sugar increased up to 71 hours for all enzymes loading. It is observed that 30ml/L enzyme loading is optimum because the reaction rate is too slow for the lower enzyme concentration again the 40ml/L enzyme comparatively less-efficient than 30ml/L enzyme loading.

3.3. Effect of pH and Temperature

Hydrolysis reactions are carried out simultaneously at different pH (4, 5, 6, 7, and 8) and at different temperature (25°C, 30°C, 35°C, 40°C, 45°C) with NH₄OH pre-treated fern (pteris) but the other parameters were kept constant, 10ml/L enzyme and 2.5gm/L substrate having particle size 45-63 μm. The result were given in the Table 2 which indicates the optimum pH is 7 and the Optimum temperature is 35°C.

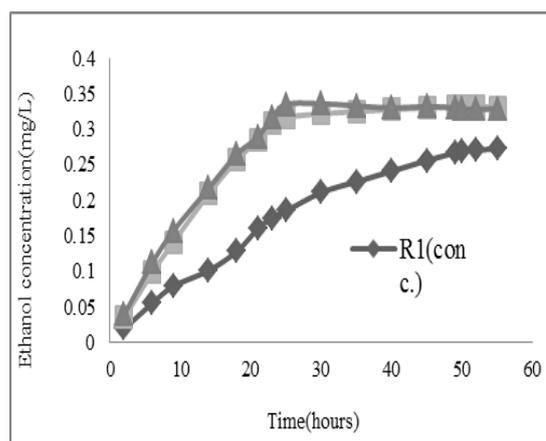
Table 2. Detection of optimum pH and temperature

pH	Glucose concentration (mg/L)	Temperature (°C)	Glucose concentration (mg/L)
4	0.450mg/L	25	0.615mg/L
5	0.489mg/L	30	1.023mg/L
6	0.589mg/L	35	1.612mg/L
7	0.615mg/L	40	1.351mg/L
8	0.577mg/L	45	1.033mg/L

3.4. Study at Optimum Condition

After optimizing the conditions (NH₄OH treatment as pre-treatment; particle size 45-63 μm; substrate loading 2.5gm/L; enzyme loading 30ml/L; pH 7; and temperature 35°C) for Enzymatic hydrolysis of pteris a further reaction was studied and found the optimum sugar concentration 1.7625mg/L.

3.5. Fermentation of the Reducing Sugar Solution

**Figure 4.** Observation the fermentation reaction rate of the reducing sugar. [R1 = 0.025gm ; R2 = 0.05gm ; R3 = 0.075gm]

After separating the sugar solution from the remaining unreacted particle using filter and decolourising with activated carbon the fermentation reaction was carried out with the *Saccharomyces cerevisiae* (Baker's yeast). For this purposes 0.025gm, 0.05gm, and 0.075gm of the *Saccharomyces cerevisiae* was added in three reactor flask having 75ml of the 1.7625mg/L concentrated reducing sugar solution. The other parameters were maintained

constant pH = 7, and Temperature = 25°C. The data collected time to time and found the total concentration of the ethanol at 50 hours later is 0.333mg/L, which was the maximum concentration of ethanol for the study found for 0.075gm of yeast, and the others are 0.27mg/L for 0.025gm yeast and 0.329mg/L for 0.05gm yeast which were seen in the [Figure 4](#).

4. Conclusion

This work is very significant because in the future the world's need much renewable energy to maintain the world's energy crisis and to protect the Environment. In this study a new hemicellulosic biomass source (pteris) is introduced into the renewable energy section which is economically less important and mostly available. This work suggests that the NH₄OH pre-treatment is the best among the NH₄OH, NaOH, and H₂SO₄ treatment for pteris. Again at the optimum condition of hydrolysis we get the optimum sugar concentration 1.7625 mg/L. and then performing fermentation with *Saccharomyces cerevisiae* (Baker's yeast) at 25°C temperature, PH = 7, we get 0.333mg/L ethanol which indicates that 0.1332mg of ethanol can be produce from 1gm of pteris where the conversion of reducing sugar to ethanol is 20% approximately in the optimum reaction condition.

Acknowledgement

This work was done under the full financial support of SUST research centre under the research grant 2013.

References

- [1] Omer AM, the Energy Crisis, the Role of Renewable and Global Warming Greener, "*Journal of Environment Management and Public Safety*," Vol. 1 (1), 038-070, 2012.
- [2] Suleiman LN, Renewable Energy as a Solution to Nigerian Energy Crisis, "*VUAS*," 13-19, 2010.
- [3] Charles. EW, Biomass ethanol: technical progress, opportunities and commercial challenges, "*Annul. Rev. Energy Environ*," 24:189-226, 1999.
- [4] DoKyoung L, Vance N, Owens AB, Peter J , Composition of Herbaceous Biomass Feedstock, SDSU, "*SGINCCR*",07, 2007.
- [5] Jenni R, "Isolation and Characterization of Lignin from steam pretreated spruce and its utilization to study cellulase adsorption on lignin" University of Helsinki, 2009, 14.
- [6] Mohammad. J.T, and Keikhosro. K, Enzyme biased hydrolysis process for ethanol from lignocellulosic materials: A Review, "*ncsu.edu/bioresources*" 2(4), 707-738.
- [7] Zheng Y, Zhongli P, Ruihong Z, Overview of biomass pretreatment for cellulosic ethanol Production, "*Int J Agric & Biol Eng*," 2(3), 51-68, 2009.
- [8] Michelle LM, Factors Effecting Ethanol Fermentation via Simultaneous Saccharification and Fermentation, Shanghai Jiao Tong University, (2011) 10.
- [9] Alexander D, Richard D, Scott G, Ryan P, Enzymatic Hydrolysis of Cellulosic Biomass for the Production of Second Generation Biofuels, "*WPI*."May, 2009.
- [10] Kim O, Magnus B and Gunnar L, A short review on SSF – an interesting process option for ethanol production from lignocellulosic feedstock, "*Biotechnology for Biofuels*" 1-7,2008.
- [11] Cowan, S. T. & Steel, K. J. "*Cowan and Steel's Manual for the Identification of Medical Bacteria*," 2nd edn. Revised by S. T. Cowan. Cambridge, UK: Cambridge University Press. 1974.
- [12] MILLER, G.L. "*Analytical Chemistry*," 1959, vol. 31, 426-428.
- [13] Adran C, Prifysgol C, "concentration of ethanol in beer and wine, Experimental Handbook,"University of Wales BANGOR, 2006.
- [14] Mark T. H, Arthur E. H., The Effect of Organosolv Pretreatment on the Enzymatic Hydrolysis of Poplar, "*Biotechnology and Bioengineering*." Vol.26, 1984.
- [15] Kristensen JB, Enzymatic hydrolysis of lignocellulose, Substrate interactions and high solids loadings, "*Forest & Landscape*," Research No. 42-2008, 2.