Annals of Agricultural & Crop Sciences

Open Access

Austin Publishing Group

Research Article

Development of a Novel Ultrasound Assisted Hydrothermal Pretreatment Strategy for the Production of Bioethanol from Chili Post-Harvest Residue

Sindhu R[1]*, Binod P[1], Mathew AK[1], Abraham A[1], Gnansounou E[2] and Pandey A[1,3]

[1]Microbial Processes and Technology Division, National Institute for Interdisciplinary Science and Technology, India

[2]Ecole Polytechnique Federale de Lausanne, Institute of Urban and Regional Sciences, Switzerland

[3]Department of Biotechnology, Center for Innovative and Applied Bioprocessing (CIAB), India

***Corresponding author:** Sindhu R, Microbial Processes and Technology Division, National Institute for Interdisciplinary Science and Technology, CSIR, Trivandrum, India

Received: November 29, 2017; **Accepted:** February 10, 2017; **Published:** February 14, 2017

Abstract

A novel ultrasound assisted hydrothermal pretreatment strategy was evaluated for the production of bioethanol from chili post-harvest residue. Various process parameters affecting pretreatment were optimized by adopting a Taguchi design. The optimum conditions of pretreatment were sonication time for 10 min, biomass loading of 25% w/w and pretreatment time for 45 min. Under optimized conditions 0. 436 g of reducing sugar per g of dry biomass (g/g) was observed. The hydrolyzate is devoid of major fermentation inhibitors like furfural, 5-hydroxymethylfurfural and organic acids like citric acid, propionic acid, succinic acid and formic acid. Fermentation of the non-detoxified hydrolyzate yielded 1.84% of ethanol. To the best of our knowledge this is the first report on ultrasound assisted hydrothermal pretreatment of chili post-harvest residue. The main highlight of this strategy of pretreatment is that the pretreated biomass can be directly used for hydrolysis without any neutralization, washing and drying.

Keywords: Pretreatment; Biomass; Bioethanol; Hydrolysis; Chili; Saccharification

Abbreviations

US: Ultra Sound; USAHTP CPHR: Ultrasound Assisted Hydrothermal Pretreated Chili Post-Harvest Residue

Introduction

Increase in consumption of fossil fuels due to industrialization and motorization of the world has resulted in fast depletion of non-renewable fuels and rising greenhouse gases concentration leads to search for alternative sources of energy [1]. Bioethanol is one of the eco-friendly alternatives to fossil fuels produced by renewable source. Lignocellulosic biomass is proved to be one of the best options for the production of alternative biofuel. Conversion of lignocellulosic biomass to bioethanol involves three major unit operations - pretreatment, hydrolysis and fermentation. One of the major problems of lignocellulose based biofuels is its cost. Several research and developmental activities are going on in this direction to make the process economically viable. The overall economy of lignocellulosic bioethanol production depends on the feed stock availability as well as the production of value added products from by-product stream [2].

Pretreatment is one of the most important steps in lignocellulosic biorefinery. Though several pretreatment strategies are available, a tailor made technology is still not available for pretreatment of specific biomass since the composition varies based on the variety and species. Each strategy has its own merits and demerits. An ideal pretreatment strategy would effectively remove lignin, no inhibitor generation and would be cost effective. Some commonly employed physical pretreatments to save energy requirements are irradiation like ultrasound, pulse electric fields and microwave. Most of the reports on physical treatment were carried out by employing hybrid strategy.

Ultrasound waves have a frequency above the human hearing range. Ultrasound (US) has been currently used as an energy source to produce fermentable sugars from biomass after pretreatment. It saves energy and produce very small sized biomass which in turn improves the enzymatic saccharification rate. US provide very high energy which will destroy microcrystalline cellulose and which in turn decrease cellulose crystallinity. The main effect of US is cavitation and acoustic streaming. Cavitation generates powerful hydro-mechanical shear forces in liquid which will disintegrate nearby particles by extreme shear force, while acoustic streaming helps in mixing and uniform distribution of US energy [3].

Several reports were available on ultrasound assisted pretreatment of lignocellulosic biomass. These includes alkaline combined ultrasonic pretreatment of corn cob [4,5], ultrasound assisted ionic liquid pretreatment of bamboo [6], ozonolysis assisted ultrasound pretreatment of sugarcane bagasse [7], ultrasound assisted acid pretreatment of chili post-harvest residue [8], surfactant assisted ultrasound pretreatment of sugarcane tops [9], ultrasound assisted Fenton pretreatment of corn cobs [10], ultrasound assisted potassium permanganate pretreatment of spent coffee waste [11], ultrasound assisted metal chloride pretreatment of sugarcane bagasse [12], ultrasound assisted ammonia pretreatment of sugarcane bagasse [13], ultrasound assisted lime pretreatment of various biomass [14] and ultrasound assisted supercritical CO_2 pretreatment of corn stalk [15]. No reports were available on ultrasound assisted hydrothermal pretreatment of lignocellulosic biomass.

The objective of the present study was to optimize various process

Citation: Sindhu R, Binod P, Mathew AK, Abraham A, Gnansounou E and Pandey A. Development of a Novel Ultrasound Assisted Hydrothermal Pretreatment Strategy for the Production of Bioethanol from Chili Post-Harvest Residue. Ann Agric Crop Sci. 2017; 2(1): 1020.

Table 1: Taguchi design for optimization of various process parameters affecting USAHTP CPHR.

Run No:	Biomass loading (% w/w)	Sonication Time (min)	Pretreatment time (min)	Reducing Sugar (g/g)
1	10	5	15	0.235
2	10	10	30	0.368
3	10	15	45	0.402
4	10	20	60	0.411
5	15	5	30	0.225
6	15	10	15	0.278
7	15	15	60	0.387
8	15	20	45	0.411
9	20	5	45	0.290
10	20	10	60	0.325
11	20	15	15	0.311
12	20	20	30	0.399
13	25	5	60	0.239
14	25	10	45	0.436
15	25	15	30	0.423
16	25	20	15	0.401

parameters affecting Ultrasound Assisted Hydrothermal Pretreatment of Chili Post-Harvest Residue (USAHTP CPHR) and utilization of the hydrolyzate obtained after enzymatic saccharification for the production of bioethanol.

Materials and Methods

Feed stock

Chili Post-Harvest Residue (CPHR) received from Virudhanagar, Tamil Nadu and India was used in this study. The samples were air dried and milled using a knife mill. Compositional analysis of native and pretreated samples was carried out by adopting NREL protocol [16].

Optimization of various process parameters affecting ultrasound assisted hydrothermal pretreatment of chili post-harvest residue

Optimization of various process parameters affecting USAHTP CPHR was carried out by adopting a Taguchi design. The experiment consists of a total of 16 runs. The details were presented in Table 1. The parameters selected were biomass (solid) loading, sonication time and pretreatment time. Parameters like biomass loading, sonication time and pretreatment time were selected at four levels.

Validations for optimized conditions of pretreatment

For the validation of the model, three confirmation experiments were carried out within the range defined previously and correlation analysis were performed based on the experimental and the predicted responses.

Enzymatic saccharification

Enzymatic saccharification of USAHTP CPHR was carried out by incubating 10% w/w of pretreated biomass with commercial cellulase (Zytek India Ltd, Mumbai, India) in 150 ml stoppered hydrolysis flasks. The enzyme loading was 30 FPU per g of pretreated dry biomass, 0.1% w/w of Tween 80 was used as surfactant, 200µl of antibiotic solution (Penicillin- Streptomycin cocktail, Hi-media, India) were added and the total reaction volume was made up to 30 ml with 0.1 M citrate buffer (pH 4.8). The samples were incubated in a shaking water bath at 50°C for 48 hrs. After incubation the samples were centrifuged to remove the residue i.e. the un-hydrolyzed biomass. Reducing sugar analysis was carried out by 3, 5-dinitrosalicylic acid method [17].

Inhibitor analysis of the hydrolyzate

The hydrolyzate obtained after enzymatic saccharification of USAHTP CPHR was centrifuged to remove the residue i.e. the unhydrolyzed biomass and filtered through 0.2µm PES membrane filters (Pall, USA) and the filtrate was evaluated for inhibitors such as furfural, 5-hydroxymethylfurfural and organic acids like citric acid, succinic acid, propionic acid, acetic acid and formic acid by HPLC. The inhibitors were analyzed using a photodiode array detector kept at 55°C. Rezex ROA columns (Phenomenex) were used with an injection volume of 10µl and flow rate was maintained at 0.6 ml/min. The concentrations of inhibitors were analyzed using the standard curve [18].

Fermentation

The hydrolyzate obtained after enzymatic saccharification of USAHTP CPHR was centrifuged at 10,000 rpm, 4°C for 10 min to remove the solids. Fermentation was carried out in stoppered bottles containing non-detoxified hydrolyzate. It was inoculated with seed culture (2% v/v) of 18 hrs old Saccharomyces cerevisiae and incubated at 30°C for 72 hrs. After fermentation, the samples were centrifuged and filtered through 0.2µm filters (Pall, USA). The ethanol was analyzed by Gas Chromatography [19].

Results and Discussion

Compositional analysis of native and pretreated chili post-harvest residue

Compositional analysis of the biomass revealed that the native biomass contains 39.95% cellulose, 17.85% hemicelluloses and 25.32% lignin. Control 1 (water alone) contains 41.05% of cellulose, 16.79% of hemicelluloses and 24.11% of lignin. Control 2 (sonication alone) contains 41.11% of cellulose, 11.11% of hemicelluloses and 23.98% of lignin. USAHTP CPHR contains 44.21% of cellulose, 10.01% of hemicelluloses and 20.21% of lignin. Mass balance analysis revealed a 35% loss of biomass during the pretreatment process. USAHTP CPHR was found to be effective in removing hemicelluloses and lignin.

Effect of different process parameters on ultrasound assisted hydrothermal pretreatment of chili post-harvest residue control experiments were carried out with water alone and sonication alone. Initial screening was carried out with 10% w/w of biomass (solid) loading. Control samples were the pretreatment were carried out with water alone gave a reducing sugar yield of 0.05 g/g, with sonication alone gave a reducing sugar yield of 0.075 g/g. USAHTP CPHR gave a reducing sugar yield of 0.230 g/g. USAHTP CPHR gave a better reducing sugar yield when compared to control samples i.e. water alone or sonication alone pretreated samples. Optimization of different process parameters affecting USAHTP of CPHR was carried out by adopting a Taguchi design.

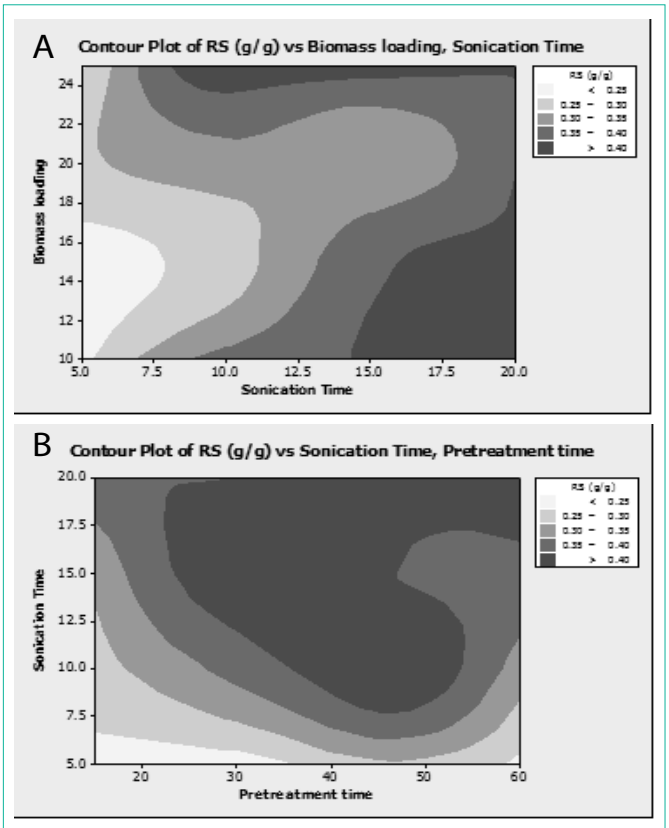

Figure 1A-B: Contour plots showing interactions of various process parameters affecting USAHTP CPHR (A) interactions between biomass loading and sonication time (B) Interactions between sonication time and pretreatment time.

The results were presented in Table 1. Maximum reducing sugar yield (0.436 g/g) was observed in Run No: 14 where the conditions of pretreatment were sonication time for 10 min, biomass loading of 25% w/w and pretreatment time for 45 min in a laboratory autoclave. Contour plots showing interactions between various process parameters affecting USAHTP of CPHR were depicted in Figure 1A-1B.

An interaction between biomass loading and sonication time is depicted in Figure 1A. At low to middle levels of biomass loading (10-20% w/w) the reducing sugar yield is high (0.40 g/g). It decreases with increase of biomass loading (20-25 % w/w). At low to middle levels of sonication time (5-12 min) the reducing sugar yield is low (0.25-0.30 g/g); it increases with increase of sonication time (15-20 min). Maximum reducing sugar yield (0.40 g/g) was observed with low to middle levels of biomass loading (10-20% w/w) and high levels of sonication time (15-20 min). An identical observation was earlier reported by Sindhu et al. [9] for surfactant assisted ultrasound pretreatment of sugarcane tops where maximum reducing sugar yield was observed at high levels of biomass loading. High biomass loading makes the process economically viable.

An interaction between sonication time and pretreatment time is depicted in Figure 1B. At low levels of sonication time (5-7 min) and low levels of pretreatment time (10-25 min) the reducing sugar yield is low (0.3 g/g). It increases with increase of sonication time

Table 2: Analysis of Variance for RS (g/g), using Adjusted SS for Tests.

Source	DF		Seq SS	Adj SS	Adj MS	F	P
Biomass loading	3		0.006153	0.006153	0.002051	2.75	0.135	
Sonication Time	3		0.058128	0.058128	0.019376	25.94	0.001	
Pretreatment time	3		0.012686	0.012686	0.004229	5.66	0.035	
Error	6		0.004482	0.004482	0.000747			
Total	15		0.081449					

S = 0.0273309 R-Sq = 94.50% R-Sq (adj) = 86.24%

and pretreatment time. Maximum reducing sugar yield (0.4 g/g) was observed at middle to high levels of sonication time (10-20 min) and pretreatment time (25-60 min). An identical observation was earlier reported by Sindhu et al. [9] for surfactant assisted ultrasound pretreatment of sugarcane tops. Sonication time have a significant impact on recovery of cellulose and cellulose recovery increased with increase of time. Contrary observation was reported by Velmurugan and Muthukumar [20] for ultrasound assisted alkaline pretreatment in sugarcane bagasse where there were high losses of cellulose and hemicelluloses component as a result of overexposure to US frequencies. In the present study since the maximum sonication time was only 20 min the loss of biomass due to prolonged exposure to US radiation was overcome. Garcia et al. [21] reported that the removal of lignin content is attributed to cavitation and oxidation of the ester and ether bonds of lignin. In aqueous solution the US waves gives rise to hydroxyl ions and hydronium ions which will react with lignocellulosic components and helps in its decay. Exposing lignocellulosic biomass to US for longer periods will results in extensive removal of lignin and hemicelluloses.

The regression coefficient for reducing sugar yield was found to be best with sonication time where the p value was 0.001. The p-value indicates the level of marginal significance within a statistical hypothesis test representing the probability of the occurrence of a given event. It is used as an alternative to rejection points to provide the smallest level of significance at which the null hypothesis would be rejected. Smaller the p-value greater is the evidence in favor of the alternative hypothesis. P-values for response less than 0.05 indicates that there is statistically significant relationship between the variables. In this model sonication time and pretreatment time are the significant factors. Other factor like biomass loading was found to be insignificant since the p value was greater than 0.05. P value less than 0.05 is found to be significant. The R^2 value explains the significance of the model. The coefficient of determination (R^2) was calculated as 94.50, indicating that the statistical model obtained was significant and can explain 94.50% variability in response and 5.5% of the variability in the responses was explained by the residue. The details were presented in Table 2.

For the validation of the model, three confirmation experiments were carried out within the range defined previously. The results were presented in Table 3. Correlation analyses were performed based on the predicted results and the experimental values. Correlation coefficient was found to be 0.916, indicating that the model developed is accurate.

Inhibitor analysis of the hydrolysate

Inhibitor profile of hydrolyzate obtained after enzymatic

Table 3: Validation of optimized conditions for USAHTP CPHR.

Biomass loading	Surfactant conc.	Incubation time	Reducing sugar (g/g)	
(% w/w)	(% w/w)	(min)	Predicted	Experimental
25	10	45	0.446	0.436
25	15	30	0.409	0.423
20	15	15	0.379	0.365

Table 4: Inhibitor profile of hydrolyzate of control samples and USAHTP CPHR.

Inhibitor	Control 1 (Sonication alone)	USAHTP CPHR
Furfural	ND	ND
5-hydroxymethyl furfural	ND	ND
Formic acid	ND	ND
Acetic acid	0.005	0.011
Citric acid	ND	ND
Succinic acid	ND	ND
Propionic acid	ND	ND

saccharification of control sample (sonication alone) and USAHTP CPHR were presented in Table 4. Major fermentation inhibitors like furfural, 5-hydroxymethylfurfural and organic acids like formic acid, citric acid, succinic acid and propionic acid were absent in control and pretreated samples. Acetic acid was present in control and USAHTP CPHR hydrolyzate. An identical observation was earlier reported by Ramadoss and Muthukumar [15] for ultrasound assisted ammonia pretreatment of sugarcane bagasse where the hydrolyzate contains less inhibitors when compared to other conventional pretreatment strategies.

Fermentation

Fermentation of the non-detoxified hydrolyzate obtained after enzymatic saccharification of USAHTP CPHR with *Saccharomyces cerevisiae* yielded 1.84% of ethanol with a fermentation efficiency of 65.27% based on the theoretical ethanol yield from glucose. This yield was obtained without any optimization of various process parameters affecting fermentation. The yield can be improved by fine tuning of various process parameters affecting fermentation. The ethanol yield is found to be higher than those reported for microwave assisted surfactant pretreatment of CPHR by Sindhu et al. [22]. The positive effect of ultrasound effect on pretreatment of rice straw for improving fermentation efficiency was reported by Belal [23] where the combined acid pretreatment with ultrasound and subsequent enzymatic saccharification yielded highest ethanol concentration of 11 g/l after seven days of fermentation with S. cerevisiae. Nikolic et al. [24] reported ultrasound assisted bioethanol production by simultaneous saccharification and fermentation of corn meal. The results indicated that ultrasound assisted pretreatment increase the ethanol concentration by 11.15% compared to control samples.

Conclusion

USAHTP was found to effective in hemicelluloses and lignin removal from CPHR. One of the main advantages of USAHTP is that the pretreated biomass can be used directly for hydrolysis without any neutralization, washing or drying. The major fermentation inhibitors like furfural, 5-hydroxymethyfurfural and organic acids like citric acid, succinic acid, formic acid and propionic acid were absent. Since the major fermentation inhibitors were absent there is no need for detoxification of the hydrolysate obtained after enzymatic saccharification. Eliminating unit operations like washing, neutralization and drying of pretreated biomass as well as detoxification of the hydrolyzate will make the process eco-friendly and economically viable. To the best of our knowledge this is the first report on USAHTP of CPHR. Fermentation of the non-detoxified hydrolysate yielded 1.84% of ethanol with a fermentation efficiency of 65.27%.

Acknowledgement

The authors are grateful to the Ministry of New and Renewable Energy, Government of India, New Delhi; Department of Science and Technology, Government of India, New Delhi; and Technology Information, Forecasting and Assessment Council, New Delhi, for the financial support provided to the Centre for Biofuels R&D, CSIR-NIIST, Trivandrum. One of the authors Sindhu R acknowledges Department of Biotechnology for financial support under DBT Bio-CARe scheme. Sindhu R and Binod P acknowledges Ecole Polytechnique Federale de Lausanne for financial support. Abraham A acknowledges Kerala State Council for Science, Technology and Environment (KSCSTE), India for providing Post-Doctoral Fellowship.

References

1. Agarwal AK. Biofuels (alcohols and biodiesel) applications as fuels for internal combustion engines. Prog Energy Combust Sci. 2007; 33: 233-271.
2. Demirbas A. The importance of bioethanol and biodiesel from biomass. Energ Sources, B Econ Plan Pol. 2008; 3: 177-185.
3. Singh R, Krishna BB, Kumar J, Bhaskar T. Opportunities for utilization of non-conventional energy sources for biomass pretreatment. Bioresour Technol. 2016; 199: 398-407.
4. Soontornchaiboon W, Kim SM, Pawongrat R. Effects of alkaline combined with ultrasonic pretreatment and enzymatic hydrolysis of agricultural wastes for high reducing sugar production. Sains Malaysiana 2016; 45: 955-962.
5. Subhedar PB, Gogate PR. Alkaline and ultrasound assisted alkaline pretreatment for intensification of delignification process from sustainable raw-material. Ultrasonics Sonochem. 2014; 21: 216-225.
6. Ninomiya K, Ohta A, Omote S, Ogino C, Takahashi K, Shimizu N. Combined use of completely bio-derived cholinium ionic liquids and ultrasound irradiation for the pretreatment of lignocellulosic material to enhance enzymatic saccharification. Chem Engg J. 2013; 215: 811-818.
7. Perrone OM, Colombari FM, Rossi JS, Moretti MMS, Bordignon SE, da Costa C, et al. Ozonolysis combined with ultrasound as a pretreatment of sugarcane bagasse: effect on the enzymatic saccharification and the physical and chemical characteristics of the substrate. Bioresour Technol. 2016; 218: 69-76.
8. Sindhu R, Binod P, Pandey A. A novel sono-assisted acid pretreatment of chili post-harvest residue for bioethanol production. Bioresour Technol. 2016; 213: 58-63.
9. Sindhu R, Kuttiraja M, Preeti VE, Vani S, Sukumaran RK, Binod P. A novel surfactant - assisted ultrasound pretreatment of sugarcane tops for improved enzymatic release of sugars. Bioresour Technol. 2013; 135: 67-72.
10. Ouyang WX, Wang W, Yuan Q, Yan A. Comparison of ultrasound-assisted Fenton reaction and dilute acid-catalysed steam explosion pretreatment of corncobs: cellulose characteristics and enzymatic saccharification. RSC Adv. 2016; 6: 76848-76854.
11. Ravindran R, Jaiswal S, Abu-Ghannam N, Jaiswal AK. Evaluation of ultrasound assisted potassium permanganate pre-treatment of spent coffee waste. Bioresour Technol. 2016; 224: 680-687.

12. Ramadoss G, Muthukumar K. Ultrasound assisted metal chloride treatment of sugarcane bagasse for bioethanol production. Renew Ener. 2016; 99: 1092-1102.

13. Ramadoss G, Muthukumar K. Ultrasound assisted ammonia pretreatment of sugarcane bagasse for fermentable sugar production Biochem Engg J. 2014; 83: 33-41.

14. Sasmal S, Goud VV, Mohanty K. Ultrasound assisted lime pretreatment of lignocellulosic biomass toward bioethanol production. Energy Fuel. 2012; 26: 3777-3784.

15. Yin J, Hao L, Yu W, Wang E, Zhao M, Xu Q, et al. Enzymatic hydrolysis enhancement of corn lignocellulose by supercritical CO_2 combined with ultrasound pretreatment Chinese J Catal. 2014; 35: 763-769.

16. Sluiter A, Hames B, Ruiz R, Scarlata C, Sluiter J, Templeton D, et al. NREL Technical Report, NREL/TP-510-42618. 2008.

17. Miller GFM. Use of dinitrosalicylic acid reagent for determination of reducing sugars. Anal Chem. 1959; 31: 426-428.

18. Raghavi S, Sindhu R, Binod P, Gnansounou E, Pandey A. Development of a novel sequential pretreatment strategy for the production of bioethanol from sugarcane trash. Bioresour Technol. 2016; 199: 202-210.

19. Sindhu R, Kuttiraja M, Binod P, Janu KU, Sukumaran RK, Pandey A. Dilute acid pretreatment and enzymatic saccharification of sugarcane tops for bioethanol production. Bioresour Technol. 2011; 102: 10915 - 10921.

20. Velmurugan R, Muthukumar K. Ultrasound-assisted alkaline pretreatment of sugarcane bagasse for fermentable sugar production: optimization through response surface methodology. Bioresour Technol. 2012; 112: 293-299.

21. García A, Erdocia X, González Alriols M, Labidi J. Effect of ultrasound treatment on the physicochemical properties of alkaline lignin. Chem Engg Process Process Intensif. 2012; 62: 150-158.

22. Sindhu R, Binod P, Mathew AK, Abraham A, Gnansounou E, Prabisha TP, et al. A novel microwave-assisted surfactant pretreatment of hili-post harvest residue for the production of bioethanol and biopolymer. J Energ Env Sustain. 2017; 2: 30-35.

23. Belal EB. Bioethanol production from rice straw residues. Braz J Microbiol. 2013; 44: 225-234.

24. Nikolic S, Mojovic L, Rakin M, Pejin D, Pejin J. Ultrasound-assisted production of bioethanol by simultaneous saccharification and fermentation of corn meal. Food Chem. 2010; 122: 216-222.

Ann Agric Crop Sci - Volume 2 Issue 1 - 2017
ISSN: 2573-3583 | www.austinpublishinggroup.com

Citation: Sindhu R, Binod P, Mathew AK, Abraham A, Gnansounou E and Pandey A. Development of a Novel Ultrasound Assisted Hydrothermal Pretreatment Strategy for the Production of Bioethanol from Chili Post-Harvest Residue. Ann Agric Crop Sci. 2017; 2(1): 1020.