SCIENTIFIC Publishing Limited

New Energy Exploitation and Application

http://ojs.ukscip.com/index.php/neea

ARTICLE

Cellulase Production by *Myceliophthora thermophila* in Solid State Fermentation and Its Utility in Saccharification of Rice Straw

Anu[1] Davender Singh[2] Vinod Kumar[3] Vijay Kumar[4] Vinay Malik[5] Bijender Singh[1,6*]

1. Laboratory of Bioprocess Technology, Department of Microbiology, Maharshi Dayanand University, Rohtak, Haryana, 124001, India
2. Department of Physics, RPS Degree College, Balana, Mahendergarh, Haryana, 123031, India
3. Department of Chemistry, Central University of Haryana, Jant-Pali, Mahendergarh, Haryana, 123031, India
4. Department of Botany, Shivaji College (University of Delhi), Ring Road Raja Garden, New Delhi, 110027, India
5. Department of Zoology, Maharshi Dayanand University, Rohtak, Haryana, 124001, India
6. Department of Biotechnology, Central University of Haryana, Jant-Pali, Mahendergarh,, Haryana, 123031, India

Received: 26 March 2022; **Accepted:** 22 April 2022; **Published Online:** 10 May 2022

Abstract: Optimization of cellulase production by thermophilic mould *Myceliophthora thermophila* BJTLRMDU3 was studied in solid state fermentation. *Myceliophthora thermophila* produced maximum cellulase (45.81 U/g DMR) at substrate to moisture ratio of 1:3 with 5-d old inoculum at water activity 0.95, ammonium sulfate (0.5%) and PEG 20000 (0.5%) at 45 °C using "one variable at a time" approach. Further supplementation of Tween-20 (0.5%) and K_2HPO_4 (0.25%) enhanced the cellulase production (56.06 U/g DMR) by *M. thermophila* in SSF. Optimization of saccharification by partially purified cellulase of *M. thermophila* (20 U), liberated maximum reducing sugars at pH 5.0 (185.56 mg/g substrate) and 60 °C (190.83 mg/g substrate) after 24 h (203.91 mg/g substrate) from sodium carbonate pretreated rice straw as compared to untreated biomass. Liberated reducing sugars were higher in sodium carbonate pretreated rice straw than untreated rice straw.

Keywords: Rice straw, *M. thermophila* BJTLRMDU3, Optimization, Cellulase, Sodium carbonate, Saccharification

1. Introduction

Renewable biofuels have been emerged as an effective ecological and economical alternative in spite of conventional fossil fuels nowadays [1-3]. In this view, lignocellulosic substrates i.e., agro-residues are highly preferred as potential and renewable carbon sources for biofuels due to release of various fermentable sugars after their hydrolysis. Utilization of agro-residues for valuable products at industrial scale somewhat decreases the problem of environment pollution due to their improper practice of disposal and open burning [2]. Cellulolytic enzymes promote the breakdown of cellulosic

*Corresponding Author:
Bijender Singh,
Laboratory of Bioprocess Technology, Department of Microbiology, Maharshi Dayanand University, Rohtak, Haryana, 124001, India; Department of Biotechnology, Central University of Haryana, Jant-Pali, Mahendergarh,, Haryana, 123031, India;
Email: ohlanbs@gmail.com

DOI: https://doi.org/10.54963/neea.v1i2.42

fraction of lignocellulose into monomeric (glucose) and oligomeric sugars. Rice straw is generated in huge amount in India at the time of harvesting of rice crops, which is burnt in open fields due to lack of improper management system. This leads to increase in environmental pollution as well as causing health hazards [2]. Approximately 500 million tonnes of rice is produced worldwide. India ranks 2nd for rice production, produces ~ 105 million tonnes rice annually that generates ~ 160 million tonnes rice straw [4]. Therefore, utilization of rice straw as substrate for microbial growth and enzyme production is the right method for the management of agricultural residue. Furthermore, enzymatic hydrolysis of pretreated rice straw will be useful in the production of different value-added products such as biofuels, sugars, prebiotics and others [1,5].

Filamentous fungi play an important role in production of lignocellulolytic enzymes at commercial scale as compared to bacteria [6,7]. Thermostable enzymes from filamentous fungi especially in solid state fermentation (SSF) are gaining enormous importance at industrial scale as they are stable under adverse environmental conditions [6,8]. *M. thermophila*, a thermophillic mould effectively utilizes the lignocellulosic substrates for the production of lignocellulolytic enzymes [5]. Fungal cellulolytic enzymes have a wide range of biotechnological applications in biomass refining, biofuels, food, paper and pulp and animal feed [9]. Hence, these microbes have been favoured as ideal candidate for enzymatic conversion of agricultural residues into sugars, which can be beneficially utilized for industrial applications. But the recalcitrant structure of lignocellulosic biomass demands a low cost, less energy utilizing, technically feasible and environment-benign pretreatment strategy for lignocellulosics derived valuable products. Pretreatment usually modifies the lignocellulosic linkages to promote the liberation of sugars for respective biofuels [1]. Alkaline pretreatment has emerged as a simple viable effective pretreatment that removes lignin for enhanced saccharification without removal of carbohydrates. Enzymatic saccharification releases various monomeric sugars, which can be fermented into valuable products i.e., biofuels [10,11]. Present study reports the cellulase production by *M. thermophila* and its utility in saccharification of untreated and pretreated rice straw.

2. Materials and Methods

Rice straw was collected locally, washed and grinded to 1 mm to 3 mm using mixer grinder followed by drying at 50 °C before used as SSF substrate.

Microorganism and culture conditions

Thermophilic mould *M. thermophila* BJTLRMDU3 was taken from the culture collection of Laboratory of Bioprocess Technology, Department of Microbiology, Maharshi Dayanand University, Rohtak, Haryana. The mold was grown on Emerson's YpSs [12] agar medium at 45 °C as described earlier [2,5] and the culture was maintained as YpSs slant and glycerol stocks at 4 °C and −20 °C respectively.

Optimization of culture conditions for cellulase production

Cellulase production by *M. thermophila* BJTLRMDU3 was optimized by "one variable at a time" approach in SSF. 5 g rice straw (in 250 mL flasks) at substrate to moisture ratio of 1:4 (pH 5.0) was autoclaved (121 °C, 20 min) and incubated at 45 °C for 4 days under static conditions after inoculated with 4 day old fungal culture. Enzymes were extracted using distilled water (20 mL/g) having Tween 80 (0.1% v/v) at shaking conditions for 1 h. The filtered clear culture supernatant was used for cellulase activity as described earlier [2,5].

Effect of various parameters such as different moistening ratio (1:3-1:7), inoculum age (3-6 days), water activity (0.85-0.95), nitrogen sources (ammonium sulphate, ammonium nitrate, sodium nitrate, yeast extract, beef extract, and urea at 0.5% w/v), PEGs (PEG 20000, PEG 8000 and PEG 400 at 0.5% w/v), surfactants (SDS, Triton-X, Tween-80, Tween-60 and Tween-20 at 0.5% w/v) and K_2HPO_4 concentrations (0.25%-1.50% w/v) were studied.

Cellulase assay

Carboxymethyl cellulase (CMCase) assay was carried out using carboxymethyl cellulose (0.5% w/v) as substrate at 60 °C and pH 5.0 (0.1 M sodium acetate buffer) as described earlier [2,3,5]. Reducing sugars were estimated using the dinitro salicyclic acid method [13]. One enzyme unit (IU) is considered as amount of enzyme required to liberate 1 µmole of glucose per min under the assay conditions. The enzyme unit is expressed as unit per gram dry mouldy residue (U/g DMR) in SSF.

Saccharification of untreated and pretreated rice straw using fungal cellulase

Rice straw (solid loading of 10%) was pretreated with sodium carbonate (1% w/v) at 121 °C, 15 psi for 60 min followed by significant washing and dried overnight at 50 °C. Saccharification of untreated and pretreated biomass was carried out by fungal (*M. thermophila)* cellulase (20 U/g) at various pH (3.0-7.0), temperatures (30 °C-60 °C) and time interval (0 h-48 h) at 60 °C under shaking (150 rpm) conditions. Centrifugation at 10,000 rpm (11,200 × g) for 10 min was carried out to get the clear supernatant for estimation of reducing sugars.

Statistical analysis

All experiments were conducted in triplicates and their average values with standard deviation are considered.

One-way analysis of variance (ANOVA), and Student's t-test were considered for data analysis. The values of p less than 0.05 were considered significant.

3. Results and Discussion

3.1 Optimization of Cellulase Production by *M. thermophila* BJTLRMDU3

Solid state fermentation is a favourable method for cultivation of filamentous fungi for cellulase production, as it provides energy as well as physical support for mycelial growth [14]. Composition of moistening medium is an important factor for the growth of microorganism in SSF [15]. Low or high moisture level than optimum level reduces the enzyme production as high moisture limits the minimal oxygen requirement of aerobic microorganisms that negatively affects the production of enzymes. Similarly, low moisture reduces the lignin solubility and swelling of substrate, resulting in severe water stress that decreases the nutrient transfer requires for growth of microorganism resulting in decreased production of enzymes [16,17]. Results indicated that *M. thermophila* produced maximum cellulase (26.01 ± 0.78 U/g DMR) at moistening ratio of 1:3 using rice straw as substrate in SSF (Figure 1). Similarly, *S. thermophile* produced maximum cellulase at 1:2.5 moisture ratio using mixed substrate as substrate [5]. Jain et al. [18] reported maximum CMCase of 70.2 U/g at 1:3 moisture ratio using wheat bran in SSF. In contrast, *M. thermophila* BJTLRMDU3 also supported high endoxylanase production using 1:7 moistening ratio with rice straw as substrate in SSF [19].

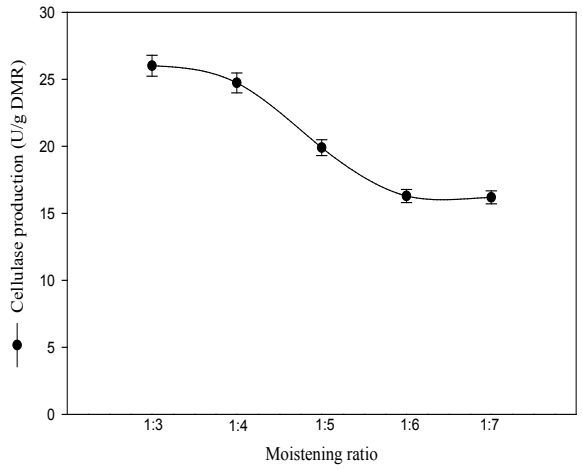

Figure 1. Effect of various moistening ratio on cellulase production by M.thermophila in SSF

Age of inoculum is also an important factor for mycelial growth, hence greatly affecting the enzyme production by fungus. Results indicated that *M. thermophila* produced maximum cellulase (42.69 ± 2.13 U/g DMR) when medium was inoculated with spore suspension 120 h old culture (Figure 2). Hemansi et al. [20] reported enhanced cellulase production (20.5 U/g) using 24 h old inoculum of *Aspergillus niger* RCKH-3 with wheat bran in SSF. Jain et al. [18] observed maximum CMCase i.e., 71.2 U/g with 72 h old inoculum of *Thermoascus aurantiacus* RCKK using wheat bran as substrate in SSF.

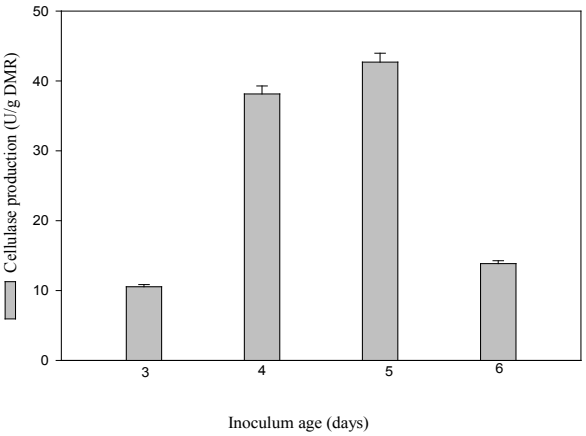

Figure 2. Effect of various inoculum age on cellulase production by *M.thermophila* in SSF

Water activity of substrate has major effect on enzyme production as well as protein stability [15]. Results indicated that *M. thermophila* secreted maximum cellulase at a_w of 0.95 (24.58 ± 0.73 U/g DMR) followed by a decline below this value (Figure 3). Similarly, *S. thermophile* also produced maximum cellulase at a_w of 0.95 using combination of wheat straw and cotton oil cake [5]. Similar findings were observed by Dahiya and Singh [19] for high endoxylanase production at a_w of 0.95 using rice straw in SSF. Sapna and Singh [15] found maximum phytase at a_w of 0.95 by the mould *A. oryzae* SBS50 in SSF.

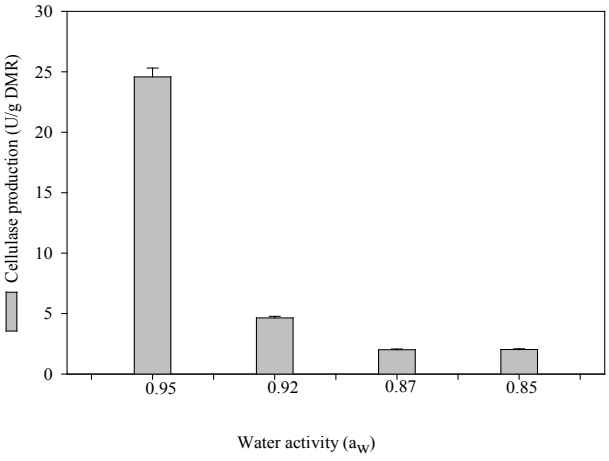

Figure 3. Effect of different water activity on cellulase production by *M.thermophila* in SSF

Among different nitrogen sources, the thermophilic mould produced maximum cellulase (45.81 ± 1.83 U/g DMR) using ammonium sulphate followed by ammonium nitrate (42.22 ± 1.68 U/g DMR) as nitrogen source (Table 1). There was 28.75% increase in cellulase production than control. Ammonium sulphate is a favourable inorganic nitrogen source for high cellulase production by *S. thermophile* [5] and *M. thermophila* ATCC 42464 [21]. *M. thermophila* ATCC 42464 also produced enhanced cellulase (5.25 FPU/g) after supplementation of 0.7% ammonium sulphate [22]. Similar results were achieved by *M. thermophila* M.7.7 using ammonium sulphate as nitrogen source [23]. Dahiya and Singh [19] observed enhanced endoxylanase by *M. thermophila* BJTLRMDU3 using ammonium nitrate as nitrogen source.

Table 1. Effect of different culture parameters on cellulase production in SSF

Culture conditions	Cellulase production (U/g DMR)
Nitrogen source (0.5%)	
Control	32.79± 0.98
Ammonium sulfate	45.81± 1.83
Ammonium nitrate	42.22± 1.68
Sodium nitrate	14.51± 0.29
Beef extract	5.14± 0.10
Yeast extract	5.31± 0.11
Urea	9.94± 0.20
PEGs (0.5%)	
Control	31.43± 0.94
PEG 400	23.46± 0.70
PEG 8000	26.74± 0.80
PEG 20000	40.80± 1.22
PEG 4000	31.35± 0.94
Surfactants (0.5%)	
Control	36.84± 1.10
SDS	29.25± 0.87
Triton-X	20.24± 0.60
Tween 20	48.24± 1.92
Tween 60	28.79± 0.86
Tween 80	22.82± 0.68
K_2HPO_4 concentration (%)	
Control	26.71± 0.80
0.25	56.06± 2.24
0.5	52.0± 2.08
1.0	42.30± 1.69
1.5	39.0± 1.17

Cellulase production was further studied using rice straw supplemented with polyethylene glycols (PEGs) of different molecular weights. Addition of PEGs not only reduced the protein adsorption but also decreased the adsorption of enzyme on lignin surface, resulting in enhanced cellulase production [24]. Another positive effect of PEG addition is to prevent the unproductive binding of various enzymes such as cellulase on lignin by forming a protective PEG layer on lignin surface via hydrogen bonding with exposed phenolic hydroxyl groups of lignin. Among the different PEGs used, supplementation of PEG-20000 resulted in maximum cellulase production (40.80 ± 1.22 U/g) by *M. thermophila* in optimized medium (Table 1). *Aspergillus niger* RCKH-3 produced maximum amount of cellulase (20.5 U/g) by supplementation of PEG 8000 to wheat bran medium in SSF [20]. Jain et al. [18] reported 67 U/g of cellulase by thermophilic fungus *Thermoascus aurantiacus* RCKK after addition of PEG-4000 as compared to others.

Surfactants enhance the microbes cell wall's permeability as well as stimulate the releasing of respective cell-bound enzymes in medium, resulting in enhanced production of enzymes [19,25]. Surfactants usually stimulate the water penetration into solid matrix, thus increases the required surface area for optimum growth of microorganism as well as promote the releasing of protein in respective medium [26]. Among all surfactants used in present study, Tween-20 supplementation enhanced the cellulase production (48.24 ± 1.92 U/g DMR) by *M. thermophila* in SSF (Table 1). Addition of Tween 20 showed 24.32% higher cellulase production as compared to control. A thermophilic *S.thermophile* produced enhanced cellulase utilizing Tween-80 in SSF [5]. Similarly enhanced cellulase production was reported by Bala and Singh [6] using Tween-80. Jain and Jain [27] reported enhanced cellulase production by *A. niger* using Tween 80 in optimized medium. Dahiya and Singh [19] reported enhancement in endoxylanase production *M. thermophila* BJTLRMDU3 using Tween 80.

Phosphorus plays an important role in microbial production of metabolites [28,29]. Results indicated that supplementation of K_2HPO_4 enhanced the cellulase production (56.06 ± 2.24U/g DMR) by *M. thermophila* BJTLRMDU3 in SSF (Table 1). Similar results were observed by Bala and Singh [6] using cane molasses medium. Salihu et al. [30] and Bibi et al. [29] found increment in endoxylanase production by supplementation of K_2HPO_4 in medium.

3.2 Saccharification of Untreated and Pretreated Rice Straw Using Fungal Cellulase

Rice straw is considered as an attractive promising substrate for microbial growth as well as production of value-added products due to its abundant availability. But the recalcitrant properties of rice straw demand an ideal pretreatment strategy to enhance the enzymatic ac-

cessibility to cellulose. In view of this, sodium carbonate pretreatment is preferred nowadays as its alkaline and deacetylating properties enhance the delignification, hence resulting in effective saccharification [2]. Reaction pH plays a significant role in forming relevant complex between enzyme and substrate, hence highly affects the enzymatic hydrolysis [31]. In the present study, it was found that maximum liberation of reducing sugars was observed at pH 5.0 in both untreated (50.00 ± 1.5 mg/g substrate) and sodium carbonate pretreated biomass (185.56 ± 9.27 mg/g substrate) using fungal (*M. thermophila)* cellulase (20 U/g) (Table 2). Liberation of reducing sugars declined at lower or higher pH than 5.0. This is due to the facts that cellulase of the mould is optimally active at pH 5.0. Previous reported studies also favoured acidic conditions for enhanced saccharification using different lignocellulosic residues [2,31,32]. Pretreatment of whole rice waste biomass with sodium carbonate followed by saccharification with 30 FPU/g at pH 5.0 liberated 352 mg/g reducing sugars [32]. Jin et al. [33] also reported enhanced saccharification in pretreated biomass at pH 4.8. Similar results were observed by Kahar [34] at pH 4.8 utilizing microbial cellulases.

Table 2. Optimization of saccharification using various parameters for enhanced liberation of reducing sugars

Conditions	Reducing sugars (mg/g substrate)	
pH	**Untreated rice straw**	**Sodium carbonate pretreated rice straw**
3.0	37.25± 1.11	144.95± 8.69
4.0	40.37± 1.21	152.47± 7.62
5.0	50.00 ± 1.50	185.56± 12.98
6.0	46.75± 1.40	174.28± 10.45
7.0	44.71± 1.34	136.52± 6.82
Temperature (°C)		
30	32.91± 0.98	113.21± 5.66
40	37.12± 1.11	157.73± 7.88
50	44.29± 1.32	173.68± 8.68
60	47.59± 1.42	190.83± 11.44
Incubation time (h)		
3	17.36± 0.52	71.09± 2.84
6	40.75± 1.22	142.84± 8.57
12	64.44± 1.93	175.78±10.54
24	180.75± 10.8	203.91±12.23
36	109.93± 5.45	194.59± 11.67
48	94.04± 3.76	169.16± 10.14

Saccharification was further carried out by fungal cellulase (20 U) at pH 5.0 at different temperatures (30 °C-60 °C). It was found that enhanced saccharification i.e., 190.83 ± 9.54 mg/g substrate observed at 60 °C in sodium carbonate pretreated biomass as compared to untreated biomass (47.59 ± 1.42 mg/g substrate) (Table 2). Alrumann [31] also reported enhanced saccharification of alkaline pretreated cellulosic date palm wastes at 60 °C. However, Khaleghian et al. [35] reported maximum liberation of reducing sugars in sodium carbonate pretreated rice straw at 45 °C. Similar results were observed by Salehi et al. [36] for enhanced liberated sugars at 45 °C.

Furthermore, enzymatic hydrolysis of untreated and pretreated rice straw was carried out at different time intervals and maximum liberation of reducing sugars was attained in pretreated biomass after 24 h (203.91 ± 10.19 mg/g substrate) that was 1.12-fold more as compared to untreated biomass (180.75 ± 9.03 mg/g substrate), followed by decline afterwards that might be due to either decline in enzymatic activity or inhibitory or negative effect of released reducing sugars on enzymatic hydrolysis [31] shown in (Table 2). Ashoor and Sukumaran [37] reported maximum liberation of reducing sugars in alkali pretreated rice straw after 48 h. Similar results were reported by Prasad et al. [38] in alkali pretreated rice straw after 48 h. In contrast, Shen et al. [39] found enhanced saccharification after 72 h in sodium carbonate pretreated rice straw. Similarly, enzymatic hydrolysis of NaOH pretreated *tritical*e straw, resulted in liberation of 184.0 mg/g sugars after 8 h [40]. A comparison of saccharification of alkaline pretreatment of rice straw is given in Table 3.

Table 3. A comparative analysis of alkaline pretreatment of rice straw carried out for enhanced saccharification

Alkaline pretreatment	Saccharification conditions	Reducing sugars (mg/g)	References
Sodium hydroxide (4%)	pH 5.0, 50 °C for 48 h	265.95	[41]
Sodium perborate (2%)	pH 4.8, 55 °C for 72 h	174.4	[42]
Sodium hydroxide (1M)	pH 4.8, 30 °C for 96 h	135.2	[43]
Sodium hydroxide (4%)	pH 5.0, 60 °C for 6 h.	145.78	[44]
Sodium carbonate (1%)	pH 5.0, 60 °C for 24 h	203.91	Present study

4. Conclusions

Thermophilic mould *M. thermophila* BJTLRMDU3 produced cellulase in SSF using rice straw. Approximately 2.15-fold increment in cellulase production was observed after optimization by "one variable at a time approach" in

SSF. Maximum saccharification by fungal cellulase was observed in sodium carbonate pretreated rice straw as compared to untreated one at pH 5.0 and 60 °C after 24 h using enzyme dose of 20 U/g substrate. Utilization of rice straw as substrate for high cellulase production followed by it saccharification using cellulase revealed the significance of research work in mitigation of environmental pollution and management of agricultural solid waste. Hence, the cellulase enzyme produced in present study can be effectively utilized in bioprocess industries for various value-added products i.e., biofuels and other.

Competing Interest

The authors declare no conflict of interest.

Acknowledgments

We acknowledge the Haryana State Council for Science and Technology, Panchkula, Haryana for the financial assistance in the form of research project (Grant no.: HSCST/R&D/2017/62) and fellowship (HSCST No. 1743, dated 12/4/2017) during the tenure of this research work. The Department of Science and Technology (No. 1196 SR/FST/LS-I/2017/4) is highly acknowledged for providing basic infrastructure facility at M.D.U., Rohtak during this research work.

References

[1] Anu, Kumar, A., Rapoport, A., et al., 2020a. Multifarious pretreatment strategies for the lignocellulosic substrates for the generation of sustainable biofuels. Renewable Energy. 160, 1228-1252.

[2] Anu, Singh, B., Kumar, A., 2020b. Process development for sodium carbonate pretreatment and enzymatic saccharification of rice straw for bioethanol production. Biomass Bioenergy. 138, 105574.

[3] Anu, Kumar, A., Jain, KK., et al., 2020c. Process Optimization for Chemical Pretreatment of Rice Straw for Bioethanol Production. Renewable Energy. 156, 1233-1243.

[4] Dahiya, S., Kumar, A., Singh, B., 2020. Enhanced endoxylanase production by *Myceliophthora thermophila* using rice straw and its synergism with phytase in improving nutrition. Process Biochemistry. 94, 235-242.

[5] Bala, A., Singh, B., 2017. Concomitant production of cellulase and xylanase by thermophilic mould *Sporotrichum thermophile* in solid state fermentation and their applicability in bread making. World Journal of Microbiology & Biotechnology. 33, 109.

[6] Bala, A., Singh, B., 2016. Cost-effective production of biotechnologically important hydrolytic enzymes by *Sporotrichum thermophile*. Bioprocess and Biosystems Engineering. 39, 181-191.

[7] Mehboob, N., Asad, M.J., Asgher, M., et al., 2014. Exploring thermophilic cellulolytic enzyme production potential of *Aspergillus fumigatus* by the solid-state fermentation of wheat straw. Applied Biochemistry & Biotechnology. 172, 3646-3655.

[8] Singh, B., 2016. *Myceliophthora thermophile* syn. *Sporotrichum thermophile*: a thermophilic mould of biotechnological potential. Critical Reviews in Biotechnology. 36, 59-69.

[9] Kuhad, R.C., Gupta, R., Singh, A., 2011. Microbial cellulases and their industrial applications. Enzyme Research.

[10] Alokika, Singh, B., 2020. Enhanced production of bacterial xylanase and its utility in saccharification of sugarcane bagasse. Bioprocess and Biosystems Engineering. pp. 1-11.

[11] Madadi, M., Tu, Y., Abbas, A., 2017. Recent status on enzymatic saccharification of lignocellulosic biomass for bioethanol production. Electronic Journal of Biology. 13, 135-143.

[12] Emerson, R., 1941. An experimental study on the life cycles and taxonomy of Allomyces. Lloydia. 4, 77-144.

[13] Miller, G.L., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry. 31, 426-428.

[14] El-Bakry, M., Abraham, J., Cerda, A., et al., 2015. From wastes to high value added products: novel aspects of SSF in the production of enzymes. Critical Reviews in Environmental Science and Technology. 45, 1999-2042.

[15] Sapna, Singh, B., 2014. Phytase production by *Aspergillus oryzae* in solid-state fermentation and its applicability in dephytinization of wheat bran. Applied Biochemistry & Biotechnology. 173, 1885-1895.

[16] Kar, S., Gauri, S.S., Das, A., et al., 2013. Process optimization of xylanase production using cheap solid substrate by *Trichoderma reesei* SAF3 and study on the alteration of behavioral properties of enzyme obtained from SSF and SmF. Bioprocess and Biosystems Engineering. 36, 57-68.

[17] Maan, P., Bharti, A.K., Gautam, S., et al., 2016. Screening of important factors for xylanase and cellulase production from the fungus *C. cinerea* RM-1 NFCCI-3086 through Plackett-Burman experimental design. BioResources. 11, 8269-8276.

[18] Jain, K.K., Dey, T.B., Kumar, S., et al., 2014. Production of thermostable hydrolases (cellulases and

xylanase) from *Thermoascus aurantiacus* RCKK: a potential fungus. Bioprocess and Biosystems Engineering. 38, 787-796.

[19] Dahiya, S., Singh, B., 2019. Enhanced endoxylanase production by *Myceliophthora thermophila* with applicability in saccharification of agricultural substrates. 3 Biotech. 9, 214.

[20] Hemansi, Gupta, R., Kuhad, R.C., Saini, J.K., 2018. Cost effective production of complete cellulase system by newly isolated *Aspergillus niger* RCKH-3 for efficient enzymatic saccharification: medium engineering by overall evaluation criteria approach (OEC). Biochemical Engineering Journal. 132, 182-190.

[21] Matsakas, L., Antonopoulou, I., Christakopoulos, P., 2015a. Evaluation of *Myceliopthora thermophila* as an enzyme factory for the production of thermophilic cellulolytic enzymes. BioResources. 10, 5140-5158.

[22] Matsakas, L., Christakopoulos, P., 2015b. Ethanol production from enzymatically treated dried food waste using enzymes produced on-site. Sustainability. 7, 1446-1458.

[23] Moretti, M.M.D.S., Bonfa, E.C., Chierotti, M.C.M., et al., 2014. Fibrolytic enzyme production of *Myceliophthora thermophila* M. 7.7. using inexpensive carbon sources and mineral nutrients. African Journal of Microbiology Research. 8, 4013-4019.

[24] Sipos, B., Szilágyi, M., Sebestyén, Z., et al., 2011. Mechanism of the positive effect of poly (ethylene glycol) addition in enzymatic hydrolysis of steam pretreated lignocelluloses. Comptes Rendus Biologies. 334, 812-823.

[25] Ramanjaneyulu, H.N.P.G., Reddy, B.R., 2016. Optimization of cellulase production by *Penicillium* sp. 3 Biotech. 6, 162.

[26] Chugh, P., Soni, R., Soni, S.K., 2016. Deoiled rice bran: a substrate for coproduction of a consortium of hydrolytic enzymes by *Aspergillus niger* P-19. Waste and Biomass Valorization. 7, 513-525.

[27] Jain, P., Jain, R.K., 2016. Enhanced cellulase production from isolated fungus *Aspergillus niger* RKJP and its application in lignocellulosic saccharification for bioethanol production. Biotechnological Research. 2, 61-68.

[28] Singh, V., Haque, S., Niwas, R., et al., 2017. Strategies for fermentation medium optimization: an in-depth review. Frontiers in Microbiology. 7, 2087.

[29] Bibi, Z., Ansari, A., Zohra, R.R., et al., 2014. Production of xylan degrading endo-1, 4-β-xylanase from thermophilic *Geobacillus stearothermophilus* KIBGE-IB29. Journal of Radiation Research and Applied Sciences. 7, 478-485.

[30] Salihu, A., Bala, S.M., Olagunju, A., 2015. A statistical design approach for xylanase production by *Aspergillus niger* using soybean hulls: optimization and determining the synergistic effects of medium components on the enzyme production. Jordan Journal of Biological Sciences. 147, 3388.

[31] Alrumman, S.A., 2016. Enzymatic saccharification and fermentation of cellulosic date palm wastes to glucose and lactic acid. Brazilian Journal of Microbiology. 47, 110-119.

[32] Saratale, G., Jung, M., Oh, M., 2016 Reutilization of green liquor chemicals for pretreatment of whole rice waste biomass and its application to 2, 3-butanediol production. Bioresource Technology. 205, 90-96.

[33] Jin, Y., Huang, T., Geng, W., et al., 2013. Comparison of sodium carbonate pretreatment for enzymatic hydrolysis of wheat straw stem and leaf to produce fermentable sugars. Bioresource Technology. 137, 294-301.

[34] Kahar, P., 2013. Synergistic effects of pretreatment process on enzymatic digestion of rice straw for efficient ethanol fermentation. Environmental Biotechnology-New Approaches and Prospective Applications. pp. 73-77.

[35] Khaleghian, H., Karimi, K., Behzad, T., 2015. Ethanol production from rice straw by sodium carbonate pretreatment and *Mucor hiemalis* fermentation. Industrial Crops and Products. 76, 1079-1085.

[36] Salehi, S., Karimi, K., Behzad, T., et al., 2012. Efficient conversion of rice straw to bioethanol using sodium carbonate pretreatment. Energy Fuels. 26, 7354-7361.

[37] Ashoor, S., Sukumaran, R.K., 2020. Mild alkaline pretreatment can achieve high hydrolytic and fermentation efficiencies for rice straw conversion to bioethanol. Preparative Biochemistry & Biotechnology. pp. 1-6.

[38] Prasad, S., Kumar, S., Yadav, K.K., et al., 2020. Screening and evaluation of cellulytic fungal strains for saccharification and bioethanol production from rice residue. Energy. 190, 116422.

[39] Shen, Z., Zhang, K., Si, M., et al., 2018. Synergy of lignocelluloses pretreatment by sodium carbonate and bacterium to enhance enzymatic hydrolysis of rice straw. Bioresource Technology. 249, 154-160.

[40] Łukajtis, R., Kucharska, K., Hołowacz, I., et al., 2018. Comparison and optimization of saccharification conditions of alkaline pre-treated *triticale* straw for acid and enzymatic hydrolysis followed by ethanol fermentation. Energies. 11, 639.

[41] Prajapati, B.P., Kango, N., 2021. Rice straw saccharification using cellulolytic cocktail from *Aspergillus tubingensis* and structure alterations studies of the wall polymer. Biomass Conversion and Biorefinery. pp. 1-15.

[42] Bera, S., Banerjee, T., Samanta, A., 2021. Evaluation of enzymatic delignification of rice straw residues for bioethanol production. International Journal of Renewable Energy Technology. 12, 99-117.

[43] Singh, B., Bala, A., Anu, et al., 2021. Biochemical properties of cellulolytic and xylanolytic enzymes from *Sporotrichum thermophile* and their utility in bioethanol production using rice straw. Preparative Biochemistry & Biotechnology. pp. 1-13.

[44] Guo, J.M., Wang, Y.T., Cheng, J.R., et al., 2020. Enhancing enzymatic hydrolysis and fermentation efficiency of rice straw by pretreatment of sodium perborate. Biomass Conversion and Biorefinery. pp. 1-10.