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Comparative Fermentable Sugar Yield from Pretreated Rice Straw by *Trichoderma reseei* and *Aspergillus tamarii* Using Plackett–Burman Method

Vikas Chandra Gupta[1*], Meenu Singh[1] and Shiv Prasad[2]

[1]*IILM-College of Engineering and Technology, Greater Noida 201306, U.P., India*

[2]*Centre for Environment Science and Climate Resilient Agriculture, IARI, New Delhi 110012, India*

ABSTRACT

A study was conducted using Plackett-Burman design to the statistical screening of seven growth medium components viz. glucose, malt, peptone, ammonium sulphate ($(NH_4)_2SO_4$), magnesium sulphate heptahydrate ($MgSO_4.7H_2O$), monopotassium phosphate (KH_2PO_4), and ferrous sulphate heptahydrate ($FeSO_4.7H_2O$) by employing microbial stains Trichoderma reesei MTCC No. 164 and Aspergillus tamarii MTCC No. 8841 on alkali pretreated and autoclaved rice straw for comparative fermentable sugar (viz., xylose and glucose) estimation. All the experimental trials were conducted in triplicate and mean value was recorded for further analysis in identification of significant factor using ANOVA analysis at α value of 0.05 and 95% confidence level. In both of A. tamarii, out of seven growth medium component used, monopotassium phosphate and glucose were found as the significant factor responsible for the release of maximum xylose concentration of 36.03 mg/g whilst ammonium sulphate was identified as a key medium component for the release of the maximum glucose concentration of 29.84 mg/g. In Treesei broth, out of seven growth medium component used magnesium sulphate heptahydrate, glucose and peptone were screened out as significant medium component responsible for the release of maximum xylose concentration of 22.15 mg/g whilst malt, ammonium sulphate and glucose were found responsible for the release of the maximum glucose concentration of 24.31 mg/g. The microbial growth media composition used was found to be more effective for A. tamarii as compared to T.reesei for production of fermentable sugars from alkali pretreated rice straw. Thus the data of the present study establishes optimum valus of selected microbial growth medium parameters responsible for enhanced yiled of fermentable sugar.

KEY WORDS: TRICHODERMA REESEI, ASPERGILLUS TAMARII, MEDIUM COMPONENT, RICE STRAW, PLACKET BURMAN DESIGN, GLUCOSE, XYLOSE.

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*Corresponding Author: vikas.gupta@iilmcet.ac.in

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INTRODUCTION

Currently, lignocellulosic biomass is being exploited extensively by many researchers across the globe for developing cleaner and sustainable energy as an alternative to the fossil fuel system (Prasad et al., 2007). Rice is an important food crop in India which is being produced at an annual rate of 106.54 million tonnes of rice and approximately 160 million tonnes of straw with a ratio of 1:1.5 for rice grain produced to straw produced (MNRE, 2017). MNRE, 2017. Energy Generation from Paddy Straw. https://mnre.gov.in › file-manager › akshay-urja › june-2017 (Singh et al., 2008). Due to lack of any suitable utilization system of this huge volume of rice straw farmers generally tend to either burn it in open field or left it in the field as a soil conditioner which ultimately affecting human health severely due to air pollution caused by burning of it and increased methane emission respectively (Singh et al., 2014).

Rice straw may be utilized for producing fuel ethanol since its structural component of the primary cell wall contains cellulose from around 32 to 47%, hemicellulose from 19 to 27% and lignin around 5 to 24% (Garrote et al. 2002; Parameswaran et al. 2010; Zamora and Crispín 1995). The cellulose and hemicellulose is an important constituent of rice straw and can be converted into simple monomeric carbohydrates such as glucose and xylose by effective pretreatment and hydrolysis methods (Sarkar et al. 2012 Cekmecelioglu and Demirci 2018). Pretreatment steps play an important role in liberating lignin and hemicellulose compounds making cellulose and hemicellulose more accessible for enzymatic conversion into fermentable sugars (Jamaldheen et al. 2019; Nigam and Singh 2011). Alkali pretreatment of rice straw has been reported as one of the most efficient method of rice straw pretreatment (Sathendra et al. 2019; Singh et al. 2011). Screening an important medium component affecting the production of fermentable sugar by Placket Buman design methods14 proves to be an efficient way as compared to one factor at the time method. Statistical significance of individual factor may be estimated using placket Burman design methods in a less number of experiments (Singh and Bishnoi 2012 Thi et al. 2018). In the present research, the medium component affecting the production of fermentable sugar was studied using Placket-Barman design method. The individual factors were screened based on the statistical significance of each medium component at alpha value of 0.05 or 95% confidence level.

MATERIAL AND METHODS

Microorganism and Inoculum Preparation: Trichoderma reesei and Aspergillus tamarii, used for the release of glucose and xylose from cellulose and hemicellulose of alkali pretreated rice straw. Both the microbial cultures were procured from Microbial Type Culture Collection Center assigned accession no., MTCC 164 and

Table 1. Assigned concentration of variables at different levels in Plackett–Burman design

S. no.	Medium component	Code	High value (+1) (% w/v)	Lower value (-1) (% w/v)
1	Malt extract	X1	0.75	0.0625
2	Ammonium sulphate	X2	1	0.1
3	Monopotassium phosphate	X3	1.25	0.083
4	Magnesium sulphate heptahydrate	X4	0.075	0.0075
5	Glucose	X5	2.5	0.0416
6	Ferrous sulphate heptahydrate	X6	0.5	0.05
7	Peptone	X7	1	0.25

Table 2. Plackett–Burman design for 7 components with coded values along with observed result for Xylose and Glucose as response

Run	X1	X2	X3	X4	X5	X6	X7	Xylose in A. tamarii broth	Response (mg/g) Xylose in T.reesei broth	Glucose in A. tamarii broth	Glucose in T.reesei broth
R1	1	-1	-1	1	-1	1	1	28.38	18.757	23.12	17.45
R2	1	1	-1	-1	1	-1	1	25.4	19.539	29.05	20.21
R3	1	1	1	-1	-1	1	-1	21.49	16.232	25.34	17.32
R4	-1	1	1	1	-1	-1	1	19.4	16.078	22.46	18.58
R5	1	-1	1	1	1	-1	-1	28.29	22.15	29.68	24.31
R6	-1	1	-1	1	1	1	-1	24.36	18.817	28.89	22.92
R7	-1	-1	1	-1	1	1	1	36.03	19.807	29.84	20.62
R8	-1	-1	-1	-1	-1	-1	-1	18.85	18.054	22.52	20.18

Table 3. Screening of significant medium component affecting fermentable sugar release in *A. tamarii* broth as per Plackett-Burman design

Fermentable sugar	Medium component level (%)	□(H)	□(L)	Difference	Effect	Mean Square	F value	P value	Confidence
Xylose	X1	107.19	103.71	3.48	0.87	1.514	5.447	0.052	94.77
	X2	105.74	105.16	0.58	0.145	0.042	0.151	0.709	29.11
	X3	107.32	103.58	3.74	0.935	1.748	6.291	0.041	95.95
	X4	104.15	106.8	-2.65	-0.663	0.878	3.159	0.119	88.12
	X5	117.46	93.44	24.02	6.005	72.12	259.502	0.007	99.33
	X6	107.19	103.71	3.48	0.87	1.514	5.447	0.052	94.77
	X7	104.47	106.43	-1.96	-0.49	0.48	1.728	0.23	76.99
Glucose	X1	103.56	98.64	4.92	1.23	3.026	1.088	0.332	66.85
	X2	90.65	111.55	-20.9	-5.225	54.601	19.641	0.003	99.7
	X3	105.21	96.99	8.22	2.055	8.446	3.038	0.125	87.51
	X4	100.43	101.77	-1.34	-0.335	0.224	0.081	0.785	21.55
	X5	114.08	88.12	25.96	6.49	84.24	30.302	0.079	92.09
	X6	110.26	91.94	18.32	4.58	41.953	2.205	0.181	81.88
	X7	109.21	92.99	16.22	4.055	32.886	11.83	0.08	91.99

MTCC 8841, at Chandigarh, India. The obtained cultures were maintained on potato dextrose agar (PDA) slants at 4^{0}C before inoculating culture with the substrate. For preparing inoculum Trichoderma reesei and Aspergillus tamarii were sub-cultured using autoclaved Malt Extract Agar medium and Czapek Yeast Extract Agar medium at 121^{0}C at 15 psi for 20 minutes, respectively. The inoculated slant cultures were incubated at 28^{0}C for 7 days after that the inoculum was obtained by adding 5 ml of sterile water to slant cultures and scrubbing the surface of slant using sterile inoculating loop wire (Kadowaki etal. 1997 Wanger et al. 2018). Substrate Preparation and Alkali Pretreatment: The rice straw was collected from the local farmer's field near to Greater Noida, U.P. The obtained rice straw was cut into pieces of size 1 cm long after that the substrate was washed with tap water to remove any dust particle attached with rice straw. The substrate was kept

Table 4. Screening of significant medium component affecting fermentable sugar release in *T.reeseibroth* as per Plackett-Burman design

Fermentable sugar	Medium component	□(H)	□(L)	Difference	Effect	Mean Square	F value	P value	Confidence level (%)
Xylose	X1	79.29	82.3	-3.01	-0.753	1.133	1.179	0.314	68.64
	X2	79.03	82.56	-3.53	-0.883	1.558	1.621	0.244	75.64
	X3	80.83	80.76	0.07	0.018	0.001	0.001	0.981	1.94
	X4	183.26	78.33	104.93	26.233	1376.288	1432.1	0	100
	X5	88.06	73.53	14.53	3.633	26.39	27.461	0.001	99.88
	X6	78.31	83.28	-4.97	-1.243	3.088	3.213	0.116	88.38
	X7	76.86	84.64	-7.78	-1.945	7.566	7.873	0.026	97.37
Glucose	X1	76.68	72.76	3.92	0.981	1.923	7.482	0.033	96.71
	X2	70.67	78.77	-8.1	-2.026	8.205	31.927	0.001	99.92
	X3	74.27	75.17	-0.9	-0.225	0.101	0.394	0.55	44.98
	X4	75.8	73.63	2.17	0.543	0.589	2.29	0.174	82.6
	X5	80.31	69.12	11.19	2.798	15.658	60.925	0	99.99
	X6	73.61	75.82	-2.21	-0.552	0.609	2.371	0.168	83.25
	X7	74.18	75.25	-1.07	-0.268	0.144	0.559	0.479	52.1

Table 5. Estimating significance of study using simple ANOVA analysis

Sum of Square (Total)	Sum of Square (Within)	Sum of Square (Between)	Degree of Freedom		F-value	P-value	Confidence level (%)
			Numerator	Denominator			
713.393	445.940	267.455	3	28	5.597	0.004	99.61

for drying in a hot air oven at 45^{0}C (Sathendra et al. 2019; Zhu et al. 1995). The dried rice straw was ground and sieved further to produce powdered raw material of size 30-50 mm. The powdered rice straw substrate was pretreated using the dilute sodium hydroxide and autoclaving after that10 gram of substrate was mixed with 80 ml of 0.5 M NaOH solution (Khanahmadi et al. 2018; Zhu et al. 2006). The mixture was autoclaved 121^{0}C at 15 psi for 10 minutes. After cooling the residue was washed thoroughly with distilled water until neutral pH is reached and oven dried at 65^{0}C and weighed for further enzymatic hydrolysis. Screening of medium component affecting the production of xylose and glucose using Plackett–Burman design: Screening of significant medium component affecting at the utmost level the microbial enzymatic hydrolysis of pretreated rice straw was performed using Plackett–Burman design (Thi et al. 2018; Plackett and Burman 1946). The following Identified independent variables including C-source, N-source, and some inorganic ions, were selected for analysis viz. Malt extract (X1), Ammonium sulphate (X2), KH_2PO_4 (X3), $MgSO_4$. $7H_2O$ (X4), Glucose (X5), $FeSO_4$. $7H_2O$ (X6) and Peptone (X7) (Mandels et al. 1974; Aggarwal et al. 2017). Each independent variable was examined at two levels, a high (+1) and a low (-1) level indicating concentration range of each parameter (Table 1). The screening experiments were conducted as per Plackett–Burman design matrix (Table 2). Inoculum at a rate of 3 ml each of Trichodermareeseiand Aspergillustamarii was transferred into separate Erlenmeyer flask of 250 ml capacity each containing the 50 ml of microbial growth medium along with 5 gram of alkali pretreated rice straw. The pH of the medium was adjusted to 6.5. The sample containing

Erlenmeyer flasks were incubated at 28^{0}C in a rotary shaker for 7 days at 160 rpm solution (Zhu et al. 2015). All experiments were carried out in the triplicate run.

Estimation of xylose and glucose: To express the accuracy, 1 ml of solution was collected from each flask at 24 hr interval for 4 days and was subjected to centrifugation at 8000 rpm at 4^{0}C in a cooling centrifuge for 10 min, the supernatant obtained was further analyzed for xylose and glucose concentrations. The amount of xylose content in the hydrolysate was estimated by phloroglucinol method (Miller 1959 Jamaldheen et al. 2019). The amount of glucose content in the hydrolysate was determined by DNS method (Eberts et al. 1979 Sorn et al. 2019). Estimation of xylose: The solution of phloroglucinol ($C_6H_6O_3$), prepared by dissolving 0.5 gram of $C_6H_6O_3$ in 100 ml of glacial acetic acid (CH_3COOH) and 10 ml of concentrated HCl. This forms a coloured reagent. 200 µl of supernatant was mixed with 5 ml of colour reagent and boiled for 5 min in boiling water bath. After cooling it, the absorbance was recorded in UV spectrophotometer at 554 nm, and the concentration of xylose was estimated against a standard xylose curve prepared (Miller 1959 Jamaldheen et al. 2019).

Estimation of Glucose: About 0.5 ml of sample was drawn from every treatment into test tubes, and the volume was made up to 3ml using distilled water. 3 ml of 3,5-dintrosalycylic acid (DNSA) reagent was added to each sample, mixed well after which the sample tubes were heated in a water bath for 5 minutes and then cooled thereafter. After cooling the absorbance was recorded in UV spectrophotometer at 540 nm and the concentration of glucose was estimated against a standard glucose curve prepared (Eberts et al. 1979; Sorn et al. 2019).

RESULTS AND DISCUSSION

Screening of medium components to enhanced fermentable sugars by Plackett–Burman design: Plackett-Burman design was used to efficiently select and screen critical nutritional variables of the growth medium of A. tamarii, and T. reesei contributed to enhanced fermentable sugars yield response from alkali pretreated rice straw by enzymatic hydrolysis under shake flask fermentation. The filamentous fungi such as Aspergillus sp. and Trichoderma sp. have been reported to produce cellulases and xylanases enzymes in a single fermentation system (Cekmecelioglu and Demirci 2018; Jampala et al. 2017). Each independent medium component has been assigned at two concentration level and as low (-1) and high (+1) coded values in the Plackett-Burman design (Table 1). The composition of the medium used for microbial growth over alkali pretreated rice straw and individual medium component affecting the enhanced release of fermentable sugar by enzymatic hydrolysis were screened with 7 medium component in 8 experimental runs in which the productivity of xylose and glucose was recorded as yield response as shown in Table 2. All the experiments were carried out as per the Plackett-Burman design matrix, and each run was conducted in triplicate and mean value was recorded against each response (Table 2). Plackett-Burman design method has been reported as an effective statistical tool in screening various factor responsible for enhanced release of xylose and glucose from lignocellulosic biomass (Singhania et al. 2007).

Effect of medium component on the yield of xylose and glucose in A. tamarii broth: Screening of nutrient medium component influencing the production of fermentable sugar especially pentose (xylose) and hexose (glucose) was analyzed and screened by Plackett-Burman experimental design. The result is presented in Table 3. Among seven nutrient components used in study, the Monopotassium Phosphate and Glucose were tested at 95.95% and 99.33% significance level respectively with p-value <0.05 found a significant factor influencing the maximum xylose concentration release of 36.03 mg/g in the A.tamarii broth in run 7 (Table 2). Monopotassium Phosphate plays an important role in A. tamarii metabolism affecting significantly enhanced release of xylose sugar in fermentation broth (Zhao et al. 2018; Maciel et al. 2008). The glucose screened as important carbon source in medium tested at a significance level of 99.33% with p-value <0.05 contributed

significantly towards the enhanced release of xylose in broth. Previous studies reported the effect of the synergistic action of glucose as a carbon source for enhanced release of xylose in A. tamarii broth when supplemented with alkali pretreated rice straw (Karunakaran et al. 2014). All other medium components were screened out as the less significant medium component for xylose release in broth found with p-value >/= 0.05 and significance level <95%. The medium component ammonium sulphate was tested at confidence level of 99.7% with p value<0.05 (Table 3) identified as an important source of inorganic nitrogen in the medium towards enhanced release of glucose in broth with a concentration of 29.84 mg/g in run 7 (Table 2) by cellulolytic enzymatic action of A. tamarii shows good agreement with previous findings (Lee 2018; Gautam et al. 2011).

Effect of medium component on the yield of xylose and glucose in T.reesei broth: Enhanced yield of xylose in the *T.reesei* broth attributed to magnesium sulphate as a significant medium component with a significance level of 100% at p value<0.05 (Table 4) indicating it as an important enzymatic cofactor (Fortkamp and Knob 2014) in releasing maximum xylose concentration of 22.15 mg/g in broth in run 5 (Table 2).

Glucose identified as a significant medium component with significance level of 99.88% at p value<0.05 for maximum xylose release by enzymatic action of T.reesei may be due to the presence of pentose sugar arabinose because of rice straw pretreatment and hydrolysis which might have enhanced enzymatic activity of *T.reesei* leading to maximum xylose release in the broth (Sorn et al. 2019; Xiong et al. 2004). Peptone with significance level tested at 97.37% at p-value <0.05 in the current study has also been identified as the best nitrogen source for the improved enzymatic action of T.reesei leading to the enhanced release of xylose in broth. Similar results were also reported previously for nitrogen source for the improved enzymatic action of T.reesei leading to the enhanced release of xylose (Gupta et al. 2018). Glucose sugar concentration was recorded as highest with a value of 24.31 mg/g in run 5 by the enzymatic action of T.reesei (Table 2) with significant medium component screened and identified as malt extract, ammonium sulphate and glucose (Table 4). Each component tested at a significance level of 97.37%, 96.71 and 99.99% respectively at p-value <0.05. The other medium component were screened out as the less significant medium component with p-value >0.05. Secretion of active cellobiohydrolase I and the endoglucanase I catalytic core domain into the culture medium were induced greatly when the Trichoderma reesei was grown on glucose-containing medium (Cekmecelioglu and Demirci 2018; Nakari and Penttilä 1995) . Enhanced released of glucose in the broth by the enzymatic action of T.reesei may also be due to an additional 15-20% carbon source of malt extract along with glucose in the medium as reported previousaly (Bagewadi et al. 2017 and Jamaldheen et al. 2019).

Ammonium sulphate has been reported as best nitrogen source in an earlier study (Guoweia et al. 2011) towards the enhanced enzymatic activity of T.reesei which finds good agreement in the current study with this factor tested at a confidence level of 99.92% (Table 4) towards the enhanced release of glucose in the broth by microbial enzymatic action.All the factors were tested at α value of 0.05 and 95 % confidence level. The significance of overall study was estimated using simple ANOVA test at the confidence level of 95%. The p-value at 95% confidence level was found to be 0.004 with F-value of 5.59 validating the experimental trail as significant (Table 5).

CONCLUSION

The medium component used for growth of T.reesei and A.tamarii were statistically screened comparatively as per the Plackett-Burman design and effect of each independent medium component on the yield of glucose and xylose concentration in the final broth were estimated. Finally, it is concluded that the microbial strain A.tamarii was found better than T.reesei for enzymatic hydrolysis of alkali pretreated rice straw leading to the enhanced release of fermentable sugar viz., xylose and glucose.

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REFERENCES

Aggarwal, Neeraj Kumar, VarshaGoyal, Anita Saini, Anita Yadav, and Ranjan Gupta. 2017. Enzymatic saccharification of pretreated rice straw by cellulases from Aspergillusniger BK01. 3 Biotech 7, no. 3: 158.

Bagewadi ZK, Mulla SI, Ninnekar HZ. 2017. Optimization of endoglucanase production from Trichoderma harzianum strain HZN11 by central composite design under response surface methodology. Biomass Conversion and Biorefinery:1-2.

Binod, Parameswaran, RaveendranSindhu, Reeta Rani Singhania, SurenderVikram, Lalitha Devi, SatyaNagalakshmi, Noble Kurien, Rajeev K. Sukumaran, and Ashok Pandey. 2010. Bioethanol production from rice straw: an overview. Bioresource technology 101, no. 13: 4767-4774.

Cekmecelioglu, D. and Demirci, A., 2018. Evaluating fungal co-production of cellulase and xylanase enzymes at shake-flask scale using distillers dried grains with solubles (DDGS) and its validation in benchtop fermenters. In 2018 ASABE Annual International Meeting (p. 1). American Society of Agricultural and Biological Engineers.

Eberts, Thomas J., R. H. Sample, M. R. Glick, and G. H. Ellis. 1979. A simplified, colorimetric micromethod for xylose in serum or urine, with phloroglucinol. Clinical Chemistry 25, no. 8: 1440-1443.

Fortkamp D, Knob A. 2014. High xylanase production by Trichoderma viride using pineapple peel as substrate and its apllication in pulp biobleaching. African Journal of Biotechnology;13(22).

Garrote, Gil, HerminiaDomí nguez, and Juan C. Parajó. 2002. Autohydrolysis of corncob: the study of non-isothermal operation for xylooligosaccharide production. Journal of Food Engineering 52, no. 3: 211-218.

Gautam SP, Bundela PS, Pandey AK, Khan J, Awasthi MK, Sarsaiya S. 2011. Optimization for the production of cellulase enzyme from municipal solid waste residue by two novel cellulolytic fungi. Biotechnology research international.;2011.

Guoweia S, Man H, Shikai W, He C. 2011. Effect of some factors on production of cellulase by Trichoderma reesei HY07. Procedia Environmental Sciences. 1;8:357-61

Gupta R, Kuhad RC, Saini JK. 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. 15;132:182-90.

Jamaldheen, S.B., Thakur, A., Moholkar, V.S. and Goyal, A., 2019. Enzymatic hydrolysis of hemicellulose from pretreated Finger millet (Eleusine coracana) straw by recombinant endo-1, 4-β-xylanase and exo-1, 4-β-xylosidase. International journal of biological macromolecules.

Jampala P, Tadikamalla S, Preethi M, Ramanujam S, Uppuluri KB. Concurrent production of cellulase and xylanase from Trichoderma reesei NCIM 1186: 2017. enhancement of production by desirability-based multi-objective method. 3 Biotech. 1;7(1):14.

Kadowaki, Marina K., Cristina GM Souza, Rita CG Simão, and Rosane M. Peralta. 1997. Xylanase production by Aspergillus tamarii. Applied biochemistry and biotechnology 66, no. 2: 97-106.

Karunakaran S, Saravanan A, Dhanasekaran S, Senbagam D, Kumar BS. 2014. Xylanase Production from Aspergillus niger. International Journal of Chem Tech Research. 6(9):4207-11.

Khanahmadi, M., Arezi, I., Amiri, M.S. and Miranzadeh, M., 2018. Bioprocessing of agro-industrial residues for optimization of xylanase production by solid-state fermentation in flask and tray bioreactor. Biocatalysis and agricultural biotechnology, 13, pp.272-282.

Lee, N.K., 2018. Statistical optimization of medium and fermentation conditions of recombinant Pichia pastoris for the production of xylanase. Biotechnology and bioprocess engineering, 23(1), pp.55-63.

Maciel GM, de Souza Vandenberghe LP, Haminiuk CW, Fendrich RC, Bianca BE, da Silva Brandalize TQ, Pandey A, Soccol CR. 2008. Xylanase production by Aspergillus niger LPB 326 in solid-state fermentation using statistical experimental designs. Food Technology and Biotechnology. 1;46(2):183.

Mandels, Mary, Lloyd Hontz, and John Nystrom. 1974. Enzymatic hydrolysis of waste cellulose. Biotechnology and Bioengineering 16, no. 11: 1471-1493.

Miller, Gail Lorenz. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry 31, no. 3: 426-

428.
Nakari-Setälä T, Penttilä M. 1995. Production of Trichoderma reesei cellulases on glucose-containing media. Applied and Environmental Microbiology. 1;61(10):3650-5.
Nigam, Poonam Singh, and Anoop Singh. 2011. Production of liquid biofuels from renewable resources. Progress in energy and combustion science 37, no. 1: 52-68.
Plackett, Robin L., and J. Peter Burman. 1946. The design of optimum multifactorial experiments. Biometrika: 305-325.
Prasad, S., Anoop Singh, and H. C. Joshi. 2007. Ethanol as an alternative fuel from agricultural, industrial and urban residues Resources, Conservation and Recycling 50, no. 1: 1-39.
Sarkar, Nibedita, Sumanta Kumar Ghosh, SatarupaBannerjee, and KaustavAikat. 2012. Bioethanol production from agricultural wastes: an overview. Renewable energy 37, no. 1: 19-27.
Sathendra, E.R., Baskar, G., Praveenkumar, R. and Gnansounou, E., 2019. Bioethanol production from palm wood using Trichoderma reesei and Kluveromyces marxianus. Bioresource technology, 271, pp.345-352.
Singh, Anita, and Narsi R. Bishnoi. 2012. Optimization of ethanol production from microwave alkali pretreated rice straw using statistical experimental designs by Saccharomyces cerevisiae. Industrial Crops and Products 37, no. 1: 334-341. from algae: an answer to debatable land based fuels. Bioresource technology 102, no.
Singh, Anoop, Poonam Singh Nigam, and Jerry D. Murphy. 2011. Renewable fuels 1: 10-16.
Singh, Jagtar, B. S. Panesar, and S. K. Sharma. 2008. Energy potential through agricultural biomass using geographical information system—A case study of Punjab. Biomass and Bioenergy 32, no. 4: 301-307.
Singh, Renu, Sapna Tiwari, Monika Srivastava, and Ashish Shukla. 2014. Microwave-assisted alkali pretreatment of rice straw for enhancing enzymatic digestibility. Journal of Energy.2312 33 41
Singhania RR, Sukumaran RK, Pandey A. 2007. Improved cellulase production by Trichoderma reesei RUT C30 under SSF through process optimization. Applied Biochemistry and Biotechnology. 1;142(1): 60-70.
Sorn, V., Chang, K.L., Phitsuwan, P., Ratanakhanokchai, K. and Dong, C.D., 2019. Effect of microwave-assisted ionic liquid/acidic ionic liquid pretreatment on the morphology, structure, and enhanced delignification of rice straw. Bioresource technology, 293, p.121929.
Thi Nguyen, H.Y. and Tran, G.B., 2018. Optimization of Fermentation Conditions and Media for Production of Glucose Isomerase from Bacillus megaterium Using Response Surface Methodology. Scientifica, 2018.
Wagner, A.O., Lackner, N., Mutschlechner, M., Prem, E.M., Markt, R. and Illmer, P., 2018. Biological pretreatment strategies for second-generation lignocellulosic resources to enhance biogas production. Energies, 11(7), p.1797.
Xiong H, Turunen O, Pastinen O, Leisola M, Von Weymarn N. 2004: Improved xylanase production by Trichoderma reesei grown on L-arabinose and lactose or D-glucose mixtures. Applied microbiology and biotechnology. 1;64(3):353-8.
Zamora, R., and JA Crispín Sánchez. 1995. Production of an acid extract of rice straw. Actacientificavenezolana 46, no. 2: 135-139.
Zhao, C.H., Liu, X., Zhan, T. and He, J., 2018. Production of cellulase by Trichoderma reesei from pretreated straw and furfural residues. RSC advances, 8(63), pp.36233-36238.
Zhu, Shengdong, Wenjing Huang, Wangxiang Huang, Ke Wang, Qiming Chen, and Yuanxin Wu. 2015. Pretreatment of rice straw for ethanol production by a two-step process using dilute sulfuric acid and sulfomethylation reagent. Applied energy154: 190-196.
Zhu, Shengdong, Yuanxin Wu, Ziniu Yu, Cunwen Wang, Faquan Yu, Siwei Jin, Yigang Ding, Ru'an Chi, Jintao Liao, and Yan Zhang. 2006. Comparison of three microwave/chemical pretreatment processes for enzymatic hydrolysis of rice straw. Biosystems engineering 93, no. 3: 279-283.
Zhu, Shengdong, Yuanxin Wu, Ziniu Yu, Jintao Liao, and Yan Zhang. 2005. Pretreatment by microwave/alkali of rice straw and its enzymic hydrolysis. Process Biochemistry 40, no. 9: 3082-3086.