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EVALUATION OF PROCESS PARAMETERS FOR ENZYMES PRODUCTION FROM *TRICHODERMA* SP. SH2 USING PINE NEEDLES BIOMASS UNDER SOLID STATE FERMENTATION

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ABSTRACT

Various optimization steps by using classical one factor at a time approach were carried out to make the production of hydrolytic enzyme cost effective and commercially viable from a potent fungal strain *Trichoderma* sp. SH2 under solid state fermentation using pine needles biomass as a substrate. The parameters i.e. temperature, moisture: substrate ratio, incubation days and pH were studied. After optimization appreciable amount of enzymes and reducing sugars i.e. laccase (3.75 U/g), cellulase (27.45 U/g), xylanase (350.17 U/g) and reducing sugars (36.90 mg/) has been achieved by using untreated pine needle biomass.

Keywords: Enzymes, Pine needles, Fermentation, *Trichoderma* sp. SH2

Introduction

Lignocellulosic biomass is a promising renewable resource for attaining value added products. (Acharya *et al*., 2010). Many microorganisms are capable of producing a variety of lignocellulolytic enzymes i.e. laccase, cellulase and xylanase which are responsible for the biodegradation of lignocellulosic wastes (Barakat *et al*., 2014). Biotechnology operations for enzyme production are potential alternative proposition for the future (Mishra *et al.,* 2016). Moreover, these wastes may contain significant concentrations of soluble carbohydrates, which are efficient in the production of ligninolytic enzymes (Elisashvili *et al*., 2009). These enzymes are strongly inhibited by the presence of cellobiose (Massadeh *et al*., 2001). They are produced by living organisms to increase the rate of a chemical reactions required for life (Li *et al.,* 2012). Being rich in cellulase, xylanase and laccase, lignocellulolytic fungi serve as a substrate for biodegradation of pine needles for the production of biofuels besides its safe disposal helping to solve the burning issue of global warming (Nagraj *et al*., 2014). Therefore, the aim of this study was to investigate the performance of the potential strain *Trichoderma sp.* SH2 isolated from rotten wood in producing ligninocellulases (laccase, cellulase and xylanase) using pine needles as substrates under sold state fermentation mode.

Materials and Methods

Collection of pine needles

The lignocellulosic pine needles were collected from different coniferous forests of Himachal Pradesh i.e. Solan, Sirmour, Kasauli, Narag, Nauni, Sultanpur and Dharampur and chipped in to 2.0 mm mesh size.

Microwave pretreatment

Pine needle biomass was subjected to microwave irradiation at 600W for 3min.

Enzymes production and extraction (Bollag and Edelstein, 1991)

10 % of inoculum of *Trichoderma* sp. SH2 was added to the 5 gram of biomass and kept for incubation in the static condition. After incubation, 50 ml of sodium citrate buffer (0.005M, pH 5.5) was added in 250 ml Erlenmeyer flask. The contents were kept in the shaker for 1 h at 120 rpm and then filtered and centrifuged at 10,000 rpm for 10 min at 4°C. The supernatant was collected for further studies.

Effect of Moisture level

Different substrate: moisture ratios i.e. 1:2, 1:3, 1:4, 1:5 and 1:6 were studied for enzyme production at 30°C for 7 days.

Effect of temperature

Temperature ranges i.e. 20°C, 25°C, 30°C, 35°C, 40°C and 45°C for 7 days.

Effect of Incubation time

Different incubation days ranging from 3, 5, 7, 10, 12 days at 30 were studied.

Effect of pH

pH ranging from 4.5, 5.0, 5.5, 6.0 and 6.5 were used in the study.

Enzyme Assays

Laccase assay

Laccase assays were performed as per Rehan et al., method (Rehan *et al*., 2016).

Cellulase assay

The sub-enzymes CMCase and FPase were measured by following standard method of Reese and Mandel (Reese and Mandel, 1963). For β-glucosidase activity the standard method of Berghem and Petterson was followed (Berghem and Petterson, 1973).

Xylanase Assay

Quantitative assays were performed for xylanase assay by applying Miller's method (Miller, 1959).

Reducing sugars

Reducing sugars were estimated by using DNSA method (Miller, 1959).

Protein assay

Protein estimation was done by using standard protocol of Lowry et al., 1951 (Lowry *et al*., 1951).

Results and Discussion

Effect of Temperature

Effect of temperature on laccase, cellulase, xylanase and reducing sugars as well as biodegradation index from *Trichoderma sp.* SH2 was studied using untreated and pretreated pine needles biomass. Maximum laccase activity was observed at 30°C i.e. 2.82 with specific activity i.e. 0.01U/mg respectively, whereas least laccase enzyme production for was found at 45°C i.e. 0.60U/g with specific activity i.e. 0.02 U/mg respectively (table 1). Maximum cellulase activity was shown at 30°C i.e. 23.10 U/g with 0.82 U/mg specific activity whereas least cellulase production was found at 45°C i.e. 12.39 U/g with 0.50 U/mg specific activity respectively (table 1). Maximum xylanase activity for untreated biomass is 268.97 U/g and least xylanase activity of 106.89 U/g with specific activity 4.39 U/mg was observed at 45°C as shown in table 1.

Table 2 depicted the effect of temperature on pretreated pine needles biomass. Maximum laccase activity i.e. 2.72U/g with specific activity i.e. 0.09U/mg was observed at 30°C and least activity was recorded at 45°C i.e. 0.13U/g**.** The maximum cellulase activity was observed at 30°C i.e. 20.70 U/g with specific activity i.e. 0.71 U/mg specific activity (table 2). On the other hand, the maximum xylanase activity was observed at 30°C i.e. 265.63 U/g with 9.22 U/mg specific activity and least activity was reported at 45°C i.e. 97.65 (table 2). Microorganisms grow slowly at a temperature below or above the normal temperature because of reduced rate of cellular production. The reduction in enzyme activity at higher temperature could be due to denaturation of enzymes. Maximum enzyme activity at optimum temperature may be due to the faster metabolic activity and increase in protein content and extracellular enzyme production in culture supernatant (Sharma and Sharma, 2016).

Effect of substrate: moisture ratio

The data revealed the difference in laccase, cellulase and xylanase production at different moistening agent i.e. 1:2, 1:3, 1:4, 1:5 and 1:6. Maximum laccase activity using untreated pine needles biomass was observed i.e. 3.45 U/g with specific activity 0.10 U/mg at 1:4 whereas least laccase production was found at 1:2 i.e. 0.89 U/g (Table 3). The maximum cellulase was found at 1:4 i.e. 27.90 U/g. Whereas least cellulase production was found i.e. 20.40 U/g at 1:6 substrate moisture level. The maximum xylanase activity was 279.30 U/g with specific activity 8.54 U/mg at 1:4 and least xylanase activity was found i.e. 234.75 U/g with specific activity 8.28 U/mg at 1:6.

Table 4 depicted the effect of substrate: moisture ratio on pretreated pine needles biomass. Maximum laccase activity i.e. 3.16 U/g with specific activity of 0.12 U/mg at 1:4 substrate moisture level was observed and least activity was recorded at 1:2 i.e. 0.39 U/g. The maximum cellulase activity was found at 1:4 i.e. 27.00 U/g with specific activity of 1.04 U/mg and least activity was observed i.e. 18.75U/g with specific activity 0.80 at 1:2 substrate moisture level. In case of xylanase, the maximum xylanase activity using pretreated pine needles biomass at 1:4 substrate moisture level i.e. 277.95 U/g with specific activity 10.77 U/mg at 1:5 and least activity was reported at 1:2 substrate moisture i.e. 228.15 U/g.

As concerned the reducing sugar production, maximum reducing sugars from untreated pine needle biomass was 26.10 mg/g at 1:4 was observed while in pretreated pine needles biomass maximum reducing sugars of 32.65 mg/g at 1:4 was recorded (table 3 and 4). Moisture is essential for growth of microorganisms, therefore in the present study, depending upon the specific characteristics water activity (a_w) requirement of all different selected strains, a variation in substrate: moisture ratio has been exhibited (Sharma et al., 2019).

Effect of incubation time

The different incubation days i.e. 3, 5, 7, 10 and 12 days were used for assessing the enzyme activity. Effect of incubation days on *Trichoderma sp.* SH2 for laccase enzyme production has been shown in table 5 and 6. Maximum laccase activity was observed i.e. 3.45 U/g with specific activity 0.12 at 10 days, whereas least laccase enzyme production was found 0.27 U/g at 3 days with specific activity 0.02 U/mg.

The maximum cellulase activity for untreated biomass i.e. 27.45 U/g with specific activity 1.08 U/mg at 7 days, whereas least cellulase production was found i.e. 20.40 U/g with specific activity of 0.83 U/mg at 3 days. In case of xylanase enzyme, the maximum i.e. 293.10 U/g of xylanase was observed at 10 days and least xylanase activity was found i.e. 253.50 U/g at 3 days.

Table 6 depicted the effect of incubation days on hydrolytic enzymes production using pretreated pine needles biomass. Maximum laccase activity was observed i.e. 3.30 U/g with specific activity i.e. 0.01 U/mg at 10 day and least activity was found i.e. 0.30 U/g at 12 days. The

maximum cellulase activity was found at 7 days i.e. 25.80U/g at 12 days and least activity was observed i.e. 19.20 U/g at 12 days of incubation. Where as maximum xylanase activity was found at 7 days i.e. 289.50 U/g and least activity was observed i.e. 227.25 U/g at 3 days. In case of reducing sugar production, maximum reducing sugars from untreated pine needle biomass i.e. 36.75 mg/g were obtained after 7 days of incubation whereas in pretreated pine needles biomass maximum reducing sugars were observed 36.60 mg/g. The decline in the activity afterwards could be due to various reasons. A decline in the enzyme afterwards may be because of proteolysis or due to depletion of nutrients available to the strain, causing a stressed microbial physiology resulting in an in activation of enzyme.

Effect of pH

The data in Table 8 and 9 revealed the difference in laccase, cellulase and xylanase production at varying pH i.e. 4.5, 5, 5.5, 6, 6.5 pH. Effect of pH for laccase enzyme production using untreated biomass has been shown in Table 7. Maximum laccase activity was observed i.e. 3.75 U/g at 6.0 pH, whereas least laccase enzyme production was found i.e. 1.80 U/g at 4.5. The maximum cellulase activity of 27.45 U/g with specific activity 1.03 U/g at 6.0 pH, whereas least cellulase production was found 21.45 U/g at 4.5. As concerned xylanase activity, the maximum was observed at 5.5 pH i.e. 350.17 U/g and least xylanase activity i.e. 209.26 U/g at 6.5 pH was observed. On the other hand, Table 8 depicted the effect of pH on pretreated pine needles biomass. Maximum laccase activity was recorded i.e. 3.60 U/g with specific activity i.e. 0.14 U/ mg at 6.0 pH.

Table 8 also showed maximum cellulase activity for pretreated pine needles biomass i.e. 25.50 U/g with specific activity 0.97 U/ mg at 6.0 pH U/mg and least activity was observed i.e. 20.85 U/g at 4.5 pH. Maximum xylanase activity of 335.25 U/g with specific activity 12.99 was observed at 5.5 pH. On the other hand, maximum reducing sugars of 36.90 mg/g was achieved at 5.5 pH (table 7) whereas in pretreated pine needles biomass, maximum reducing sugars i.e. 32.10 mg/g at 5.5 pH were observed (table 8). Generally most of the fungi prefer to act favorably at lower pH. In this study, optimum pH for maximum enzyme and sugar production had been noticed between 5 to 6.0 thus confirming the acidophilic nature of these fungi. Each microorganism possesses a specific pH range for its growth and activity. The extracellular pH has a strong influence on the pathways of metabolism and product formation by microorganism. Changes in the external pH alter the ionization of nutrient molecules and reduce their availability to the organism thus lowering their overall metabolic activity (Sharma and Sharma, 2016).

As the Fig (3 and 4) revealed the overall increase after optimization of different process parameters i.e. temperature, substrate: moisture ratio, incubation days and pH in untreated and pretreated pine needles biomass for extracellular enzymes

activity i.e. 2.82 U/g to 3.75U/g with % increase of 75.20 (laccase), 23.10 U/g to 29.70 U/g with % increase of 77.77 (cellulase), 268.97 U/g to 350.17 U/g with % increase of 76.81(xylanase) and 25.35 mg/g to 36.90 mg/g with % increase of 68.69 (reducing sugars). It showed the best enzyme activity in untreated pine needles biomass over pretreated biomass under OFAT with percent increase 142.55 (cellulase), 109.26 (xylanase), 213.50 (laccase) and 140.18 (reducing sugars) therefore this potential strain *Trichoderma sp.* SH2 is highly recommended for biological pretreatment for lignocellulosic waste and for the production of industrially important enzymes.

Table1. Effect of temperature on enzymes production using untreated biomass

Temperature (◦C)	Laccase activity*	Specific activity**	CMCase activity	FPase activity	β-glucosidase activity	Total cellulase activity	Specific activity	Xylanase activity	Specific activity	Reducing sugars (mg/g)***	%	Protein mg/g	%	Biodegradation index
20	1.77	0.08	10.63	7.43	0.46	18.30	0.70	214.36	8.30	21.95	2.19	25.8	2.58	3.67
25	2.73	0.10	11.84	7.44	0.48	19.50	0.72	265.31	9.88	23.85	2.38	26.85	2.68	3.87
30	**2.82**	0.10	13.60	9.18	0.49	**23.10**	0.82	**268.97**	9.64	**25.35**	2.53	27.90	2.79	**4.05**
35	1.35	0.05	9.19	5.66	0.45	15.15	0.57	115.30	4.36	24.75	2.47	26.40	2.64	3.87
40	0.90	0.03	8.08	4.44	0.42	112.72	4.42	110.84	4.34	24.0	2.40	25.50	2.55	3.75
45	0.60	0.02	7.67	4.39	0.39	12.39	0.50	106.89	4.39	22.80	2.28	24.30	2.43	3.57
$CD_{0.05}$	0.018	0.018	0.019	0.018	0.018	0.015	0.018	4.274	0.019	0.019	0.019	0.022	0.019	0.021
S.E.	0.008	0.008	0.008	0.008	0.008	0.007	0.008	1.894	0.008	0.009	0.009	0.01	0.009	0.02

* U/g = μ moles of reducing sugars released / min / g of biomass
**Specific activity = activity/mg protein concentration
***Reducing sugars released mg/g

Table2. Effect of temperature enzymes production using pretreated biomass

Temperature (°C)	Enzymes activities													Biodegradation Index (BI)
	Laccase activity	Specific activity	CMCase activity	FPase activity	β-glucosidase activity	Cellulase activity	Specific activity	Xylanase activity	Specific activity	Reducing sugars (mg/g)	%	Protein mg/g	%	
20	2.01	0.12	10.25	6.96	0.46	17.55	1.04	162.99	9.74	21.79	2.17	16.72	1.67	2.75
25	2.58	0.09	11.20	7.23	0.47	18.75	0.66	263.42	9.29	22.35	2.23	28.30	2.83	3.94
30	**2.72**	0.09	12.30	8.28	0.49	**20.70**	0.71	**265.63**	9.22	**24.90**	2.49	28.8	2.88	**4.12**
35	0.85	0.03	9.12	5.43	0.45	14.85	0.54	127.97	4.66	24.45	2.44	27.45	2.74	3.96
40	0.23	0.008	8.06	5.27	0.44	13.50	0.51	117.25	4.44	23.55	2.35	26.4	2.64	3.81
45	0.13	0.005	7.73	4.36	0.41	12.30	0.48	97.65	3.82	22.50	2.25	25.5	2.55	3.67
$CD_{0.05}$	0.017	0.016	0.018	0.019	0.018	0.019	0.018	0.018	0.019	0.02	0.019	0.018	0.019	0.021
S.E.	0.008	0.007	0.008	0.006	0.006	0.008	0.008	0.006	0.008	0.009	0.008	0.008	0.009	0.02

Table3. Effect of substrate: moisture level on enzymes production using untreated biomass

Substrate :moisture ratio	Enzyme activities									Reducing sugars		Protein		Biodegradation Index (BI)
	Laccase (U/g)	Specific activity (U/mg)	CMCase (U/g)	FPase (U/g)	β-glucosidase (U/g)	Cellulase activity (U/g)	Specific activity (U/mg)	Xylanase (U/g)	Specific activity (U/mg)	(mg/g)	%	mg/g	%	
01:02	1.37	0.06	10.93	11.14	1.36	23.25	1.04	252.75	11.36	22.8	2.28	22.23	2.22	3.36
01:03	3.07	0.09	12.39	13.05	1.39	26.70	0.82	262.80	8.07	24.45	2.44	32.53	3.25	4.47
01:04	**3.45**	0.10	13.83	12.30	1.83	**27.90**	0.85	**279.30**	8.54	**26.10**	2.61	32.70	3.27	**4.57**
01:05	2.02	0.06	9.13	13.39	1.62	23.85	0.75	254.70	8.06	25.8	2.58	31.59	3.15	4.44
01:06	0.89	0.03	6.09	9.43	1.45	20.40	0.71	234.75	8.28	25.50	2.55	28.35	2.83	4.10
$CD_{0.05}$	0.018	0.017	0.019	0.044	0.018	0.015	0.018	0.044	0.018	0.019	0.019	0.022	0.019	0.022
S.E	0.008	0.008	0.009	0.02	0.008	0.007	0.008	0.019	0.008	0.009	0.008	0.01	0.008	0.01

Table4. Effect of substrate: moisture level enzymes production using pretreated biomass

Substrate :moisture ratio	Enzyme activities									Reducing sugars		Protein		Biodegradation Index (BI)
	Laccase (U/g)	Specific activity (U/mg)**	CMCase (U/g)	FPase (U/g)	β-glucosidase (U/g)	Cellulase activity (U/g)	Specific activity (U/mg)	Xylanase (U/g)	Specific activity (U/mg)	(mg/g)***	%	mg/g	%	
01:02	0.39	0.01	10.65	10.12	1.25	21.90	1.08	228.45	11.32	24.83	2.48	20.18	2.01	3.25
01:03	1.90	0.08	11.25 (0.75)	10.39	1.44	22.95	1.02	253.35	11.33	31.27	3.12	22.35	2.23	3.79
01:04	**3.16**	0.12	12.75	12.55	1.83	**27.00**	1.04	**277.95**	10.77	**32.65**	3.26	25.80	2.58	**4.21**
01:05	0.94	0.03	13.33	9.67	1.57	24.75	0.97	258.45	7.31	31.48	3.14	25.35	2.53	4.10
01:06	0.70	0.03	8.55	8.73	1.53	18.75	0.80	228.15	9.81	27.79	2.77	23.25	2.32	3.70
$CD_{0.05}$	0.018	0.018	0.021	0.018	0.018	0.019	0.018	0.374	0.018	0.024	0.018	0.019	0.019	0.024
S.E	0.008	0.008	0.009	0.008	0.008	0.009	0.008	0.166	0.008	0.011	0.008	0.008	0.009	0.02

Table5. Effect of incubation time on enzymes production using untreated biomass

Incubation time (days)	Enzyme activities									Reducing sugars		Protein		Biodegradation index (BI)
	Laccase (U/g)	Specific activity (U/mg)	CMCase	FPase activity	β-glucosidase activity	Cellulase Activity (U/g)	Specific activity	Xylanase activity (U/g)	Specific activity	(mg/g)	%	(mg/g)	%	
3	0.27	0.01	10.95	10.05	0.44	21.30	0.91	253.50	10.83	28.35	2.83	23.40	2.34	3.75
5	1.68	0.06	11.7	10.50	0.46	22.65	0.93	254.10	10.45	31.80	3.18	24.30	2.43	4.02
7	2.54	0.10	14.85	13.95	0.51	**29.25**	1.16	282.75	11.15	**36.75**	3.67	25.35	2.53	**4.36**
10	**3.45**	0.12	13.80	10.35	0.55	24.60	0.91	**293.10**	10.91	33.45	3.34	26.85	2.68	4.35
12	0.30	0.01	9.30	10.80	0.30	20.40	0.83	278.85	11.47	29.40	2.94	24.3	2.43	3.90
$CD_{0.05}$	0.018	0.019	0.018	0.018	0.018	0.019	0.018	0.064	0.003	0.019	0.019	0.018	0.019	0.023
S.E.	0.008	0.006	0.008	0.006	0.008	0.006	0.008	0.028	0.001	0.009	0.008	0.008	0.009	0.016

Table6. Effect of incubation time enzymes production using pretreated biomass

Incubation time (days)	Enzyme activities									Reducing sugars		Protein		Biodegradation index (BI)
	Laccase (U/g)	Specific activity (U/mg)	CMCase activity	FPase activity	β-glucosidase activity	Total cellulase activity	Specific activity	Xylanase activity	Specific activity	(mg/g)	%	(mg/g)	%	
3	0.66	0.02	10.80	9.45	0.41	20.55	0.86	239.70	10.05	25.25	2.52	23.85	2.38	3.64
5	1.64	0.06	11.40	9.75	0.46	21.60	0.88	243.45	10.01	28.35	2.83	24.3	2.43	3.84
7	1.75	0.06	13.35	13.35	0.56	**27.15**	0.97	288.30	10.38	**36.60**	3.66	27.75	2.77	**4.60**
10	**3.30**	0.11	13.20	9.90	0.55	23.55	0.78	**289.50**	9.69	33.00	3.30	29.85	2.98	4.63
12	0.30	0.01	9.45	9.30	0.45	19.20	0.67	227.25	0.96	29.85	2.98	28.35	2.83	4.32
$CD_{0.05}$	0.018	0.016	0.018	0.018	0.019	0.018	0.018	0.038	0.018	0.017	0.018	0.044	0.018	0.019
S.E.	0.008	0.007	0.008	0.008	0.009	0.007	0.008	0.017	0.008	0.008	0..008	0.019	0.008	0.009

Table 7. Effect of pH on enzymes production using untreated biomass

pH	Enzyme activities									Reducing sugars		Protein		Biodegradation Index (BI)
	Laccase (U/g)	Specific activity (U/mg)	CMCase activity	FPase activity	β-glucosidase activity	Total cellulase activity	Specific activity	Xylanase activity	Specific activity	(mg/g)	%	mg/g	%	
4.5	1.80	0.07	11.40	9.75	0.42	21.45	0.87	244.41	9.93	28.80	2.88	24.60	2.46	3.90
5	2.75	0.10	11.85	10.80	0.45	23.10	0.91	257.85	10.23	29.85	2.98	25.20	2.52	4.01
5.5	3.45	0.13	14.70	14.40	0.60	**29.70**	1.14	**350.17**	13.49	**36.90**	3.69	25.95	2.59	**4.43**
6	**3.75**	0.14	13.80	13.20	0.56	27.45	1.03	235.50	8.92	33.90	3.39	26.40	2.64	4.33
6.5	2.85	0.11	13.20	10.95	0.45	24.60	0.97	209.26	8.25	31.95	3.19	25.35	2.53	4.12
$CD_{0.05}$	0.019	0.017	0.018	0.018	0.018	0.019	0.016	0.021	0.018	0.022	0.019	0.019	0.019	0.023
S.E.	0.009	0.006	0.008	0.006	0.008	0.009	0.007	0.01	0.008	0.01	0.009	0.008	0.008	0.013

Table8. Effect of pH enzymes production by using pretreated biomass

pH	Enzyme activities									Reducing sugars		Protein		Biodegradation Index (BI)
	Laccase (U/g)	Specific activity (U/mg)	CMCase activity	FPase activity	β-glucosidase activity	Total cellulase activity	Specific activity	Xylanase activity	Specific activity	(mg/g)***	%	mg/g	%	
4.5	1.50	0.06	11.25	9.30	0.30	20.85	0.84	254.85	10.29	28.20	2.82	24.75	2.47	3.88
5	2.70	0.10	12.00	10.35	0.45	22.80	0.89	255.45	10.07	28.50	2.85	25.35	2.53	3.95
5.5	3.45	0.13	12.30	12.30	0.52	25.05	0.97	**335.25**	12.99	**32.10**	3.21	25.80	2.58	**4.18**
6	**3.60**	0.14	13.50	14.40	0.49	**28.20**	1.13	301.80	12.12	31.80	3.18	24.90	2.49	4.08
6.5	2.85	0.11	11.85	10.80	0.42	22.95	0.95	234.90	9.78	29.70	2.97	24.00	24.00	3.88
$CD_{0.05}$	0.018	0.018	0.018	0.018	0.018	0.016	0.018	0.076	0.019	0.017	0.018	0.02	0.019	0.024
S.E.	0.008	0.006	0.006	0.008	0.008	0.007	0.008	0.034	0.009	0.007	0.008	0.009	0.009	0.014

Fig1. Morphology of *Trichoderma* sp. SH2

Fig2. Enzyme production by *Trichoderma* sp. SH2 under solid state fermentation

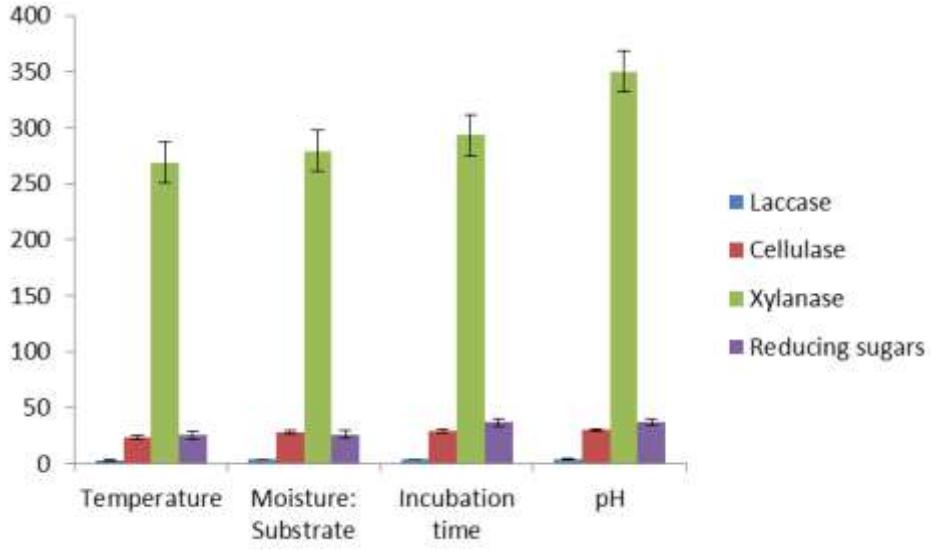

Fig3. Overall increase in enzyme activities and reducing sugars using untreated biomass after optimization of process parameters

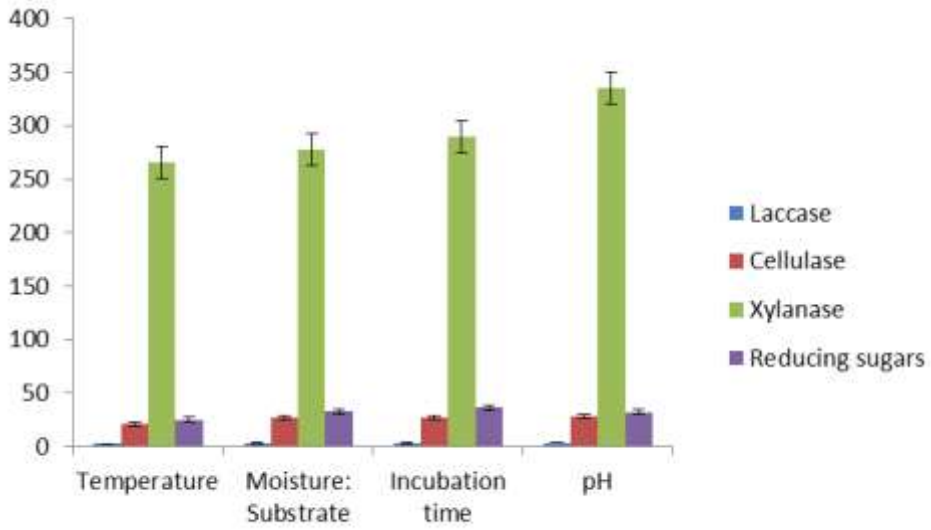

Fig4. Overall increase in enzyme activities and reducing sugars using pretreated biomass after optimization of process parameters

Conclusion

The demand for enzymes in the global market is projected to rise at a fast pace in recent years. There has been a great increase in industrial applications of hydrolytic enzymes owing to their significant biotechnological uses. Therefore the study was undertaken with aims of enhanced production of laccase, cellulase and xylanase enzymes from a potential fungal strain *Trichoderma sp.* SH2. In the present study, an attempt was made to optimize the various parameters i.e. different moisture: substrate ratio, temperature, incubation days and pH to increase production level of enzymes and reducing sugars and achieved 75.20 (laccase), 77.77 (cellulase), 76.81(xylanase) and 68.69 (reducing sugars) % increase respectively from *Trichoderma sp.* SH2.

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