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Research Article

INFLUENCE OF GROWTH PARAMETERS ON BIODELIGNIFICATION OF *EUCALYPTUS TERETICORNIS* BY *SCHIZOPHYLLUM COMMUNE*

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ABSTRACT

Pulp and paper industry use different chemicals to separate cellulose fibre from lignocellulosic biomass for papermaking. The use of sodium sulphide and chlorine during pulping and bleaching processes, have now been classified as the main sources of air and water pollution in the pulp and paper industry. To surmount these problems biological processing, offers potential opportunities for changing the industry towards more environment friendly industry. In the biological pulping process, removal of lignin can be achieved through treatment of lignocellulosic materials with white rot fungi due to their ability to degrade complex and recalcitrant organic molecules and this makes them attractive micro-organism for biodelignification. In the present study biological pre-treatment of *Eucalyptus* chips was carried out with *Schizophyllum commune*. The influence of growth parameters like incubation periods, moisture levels, media, media concentration, pH and temperature were also optimized. During the study it was found that *Schizophyllum commune* shows 14.72% lignin loss within 28 days at optimum conditions i.e. pH (6.0), temperature (25^{0}C), moisture (60%) and molasses concentration (4%). Thus the study will provide an insight to find out economically feasible conditions to commercialize biopulping on large scale.

Keywords: Biopulping, Delignification, White Rot Fungi, Lignin, Cellulose.

1. INTRODUCTION

The pulp and paper industry is one of India's oldest and core industrial sector. The socio-economic importance of paper has its own value to the country's development as it is directly related to the industrial and economic growth of the country. Fibres for paper are isolated from the wood and/or agro based raw materials using conventional mechanical or chemical methods [1]. The production of chemical pulps requires a large amount of chemicals that may have negative environmental impact and mechanical pulping requires substantial amount of energy [2-4]. Therefore, research on the development of alternative methods is still being undertaken worldwide. Biodelignification may provide new technologies for the pulp and paper industry to decrease environmental impacts and investment costs [5-7]. In this view, wood chips are treated by lignin-degrading fungi, and as a result, the chemical and energy requirement in pulping is decreased and fibre properties of the pulp are improved [8, 9]. Our objective was to study the effect of growth parameters on biodelignification of eucalyptus by *Schizophyllum commune*.

2. MATERIAL AND METHODS

2.1. Fungal Culture

The freeze dried white rot fungi *Schizophyllum commune* was obtained from Forest Pathology Division, Forest Research Institute, Dehradun. Potato Dextrose Agar plates were inoculated from the slants and incubated at 27 ± 1°C for 7 days. Active inocula from these plates were grown in a 250 ml Erlenmeyer flask containing 100 ml malt extract broth. The fungal mat was removed from the medium, suspended in sterilized distilled water and was converted into uniform suspension by using magnetic stirrer at high speed. This suspension was used to inoculate the wood samples.

2.2. Sample Preparation

The wood of plant *Eucalyptus tereticornis* was chopped in pilot plant chipper. The chips (2.0 mm – 2.3 mm thick) so obtained were dried in sunlight for 15 days to the normal moisture content. Sample was then analysed for lignin and holocellulose contents using TAPPI standard methods T 222 om-88 and Tappi Useful Method 249 respectively.

The biodelignification of eucalyptus chips was performed in petri plates, containing 50 g (Oven Dry basis). Distilled water was added to the samples in sufficient quantity to increase the moisture levels from 60 to 100% on a dry weight basis for optimum growth of the fungi. The nutrients malt extract broth and molasses solutions having different initial pH values were added at different concentration to the raw material and mixed well. Petri plates were autoclaved for 20 minutes at 121°C. The autoclaved wood chips were inoculated with mycelium suspension of *Schizophyllum commune*. The rate of mycelium application was 0.003 gm (O.D.).

Experiments were performed to obtain the best conditions for delignification by *Schizophyllum commune*. Parameters considered for obtaining the best conditions included incubation time (7, 14, 21 28, 35 and 42), moisture (60, 80, 100%), pH (4.5, 5, 5.5, 6, 6.5, 7), media (Malt Extract Broth and Molasses), media concentration (2, 4, 6, 8, 10%) and temperature (20, 25, 30, 35^{0}C). Effect of one variable on delignification was studied after keeping the other variables constant [10-12].

The initial experiment to study the extent of delignification was carried out upto 42 days in the incubator. The samples were drawn at regular interval of 7 days to monitor the rate of delignification. In the initial experiment conditions maintained were 60% moisture, 2% media dose, pH adjusted to 6 and temperature 25°C. The optimum time conditions obtained by this experiment was used in subsequent experiment to find out effect of moisture by varying it from 60 to 100% moisture level at optimized time period. The nutrients malt extract broth and molasses were added by varying concentration from 2% to 10% to find out effect of media and media concentration at optimized time period and moisture level. In other experiments pH and temperature were varied to find out the effect of these variables on delignification. The nutrient solution having different initial pH from 4.5 to 7.0 values was added to find out effect of pH on delignification at optimized time period, moisture level, media and media concentration. The effect of temperature was observed by varying it from 20^{0}C to 35^{0}C at optimized time period, moisture level, media, media concentration and pH. For the purpose of analytical studies, petri plates without inoculum were used as control. Each experiment was done in triplicate. After harvesting, each substrate was oven dried at 50 to 60^{0}C for 48 hours. Dried samples of eucalyptus were converted into dust by Willy Mill and dust passing through 40 mesh and retained over 60 mesh was used for all subsequent analytical studies. TAPPI T 222 om-88 and Useful Method 249 were used for determination of klason lignin and holocellulose respectively.

2.3. Experimental Design and Statistical Analysis

All the experiments were conducted with triplicate and were designed using a Complete Random Design (CRD). Data were subjected to Analysis of Variance (ANOVA) using the Statistical Package for Social Sciences (SPSS) version 16. The significant differences among the treatments were compared using Duncan's Multiple Range Tests (DMRT). Treatment values were represented as the mean ± SE.

3. RESULTS AND DISCUSSION

3.1. Lignin and Holocellulose Estimation

The lignin content of eucalyptus untreated chips was found to be 34.20% and the total holocellulose content was 64.38% respectively.

3.2. Effect of Time on Biodelignification

Table 1 show that lignin contents of eucalyptus chips dropped from 34.20% to 29.32% in over a period of 42 days. On a periodic basis loss in lignin observed from 7 to 42 days was 1.36% to 14.28%. Lignin loss was increased significantly with increasing incubation time upto 42 days when compared using Duncan's Multiple Range Test (DMRT). But a sharp significant increase in lignin loss was observed between the incubation periods of 21 to 28 days when it went up from 6.68% to 10.96% i.e. an increase of 4.28%. After increasing the incubation period above 28 days, rate of delignification starts decreasing. Loss in holocellulose after different incubation periods of 7 to 42 days was 0.98 – 11.62%. As observed the maximum rate of lignin degradation occurred between 21 to 28 days when the loss of holocellulose was found to be only 5.59% in the samples. After 28 days holocellulose loss percent increased rapidly from 5.59% to 9.26% thus showing a sudden increase of 3.67% which was much greater in comparison to lignin loss percent at the same time i.e. 10.96% to 12.82% registering total loss of 1.86% only. ANOVA showed significant difference between lignin and holocellulose degradation by varying incubation periods. On the basis of above results 28 days were considered as optimum incubation period for fermentation without a significant loss of holocellulose.

Table 1: Effect of Incubation Period on Lignin and Holocellulose Degradation by Schizophyllum commune

S. No.	Days	Lignin%	Lignin Loss %	Difference in Lignin Loss %	Holocellulose %	Holo Loss %	Difference in Holocellulose Loss%
1	Control	34.20±0.18^{g}	Nil	Nil	64.38±0.16^{g}	Nil	Nil
2	7	33.73±0.12^{f}	1.36	Nil	63.75±0.12^{f}	0.98	Nil
3	14	32.90±0.16^{e}	3.80	2.44	62.98±0.20^{e}	2.17	1.19
4	21	31.92±0.17^{d}	6.68	2.88	61.90±0.18^{d}	3.85	1.68
5	28	30.45±0.14^{c}	10.96	4.28	60.78±0.25^{c}	5.59	1.74
6	35	29.82±0.12^{b}	12.82	1.86	58.42±0.12^{b}	9.26	3.67
7	42	29.32±0.22^{a}	14.28	1.46	56.90±0.18^{a}	11.62	2.36
ANOVA		F computed = 867.37 P<0.05			F computed = 1472.95 P<0.05		

Means with similar superscript in columns are non-significant (P>0.05) with each other. Duncan's multiple range test with level of significance = 0.05.

3.3. Effect of Moisture on Biodelignification

The lignin loss (%) observed was significantly decreased with increasing moisture level upto 100%. It was observed that the lignin loss percent was more at 60% initial moisture level i.e. 10.96%. At the same time the loss percent of holocellulose was 5.59% due to more growth of fungi (Table 2). On the basis of these observations 60% initial moisture level was taken as optimum for treatment of chips to conduct further experiments.

Table 2: Effect of Moisture on Lignin and Holocellulose Degradation by Schizophyllum commune

S. No.	Moisture (%)	Lignin (%)	Lignin Loss (%)	Holocellulose (%)	Holocellulose Loss (%)
1	Control	34.20±0.18^{d}	Nil	64.38±0.16^{c}	Nil
2	60	30.45±0.14^{a}	10.96	60.78±0.25^{a}	5.59
3	80	30.87±0.23^{b}	9.75	60.97±0.21^{a}	5.30
4	100	31.17±0.26^{c}	8.87	61.25±0.19^{b}	4.86
ANOVA		F computed = 407.95 P<0.05		F computed = 421.61 P<0.05	

Means with similar superscript in columns are non-significant (P>0.05) with each other. Duncan's multiple range test with level of significance = 0.05.

Table 3: Effect of Media and Media Concentration on Lignin and Holocellulose Degradation by Schizophyllum commune

S. No.	Media	Media Concentration (%)	Lignin (%)	Lignin Loss (%)	Holocellulose (%)	Holocellulose Loss (%)
1		Control	34.20±0.18^{e}	Nil	64.38±0.16^{f}	Nil
2	Malt	2	30.07±0.24^{d}	12.09	60.30±0.22^{a}	6.34
3	Extract	4	28.85±0.24^{c}	15.64	61.70±0.23^{b}	4.16
4	Broth	6	28.20±0.23^{b}	17.54	62.35±0.10^{c}	3.15
5		8	27.75±0.10^{a}	18.86	62.75±0.12^{d}	2.53
6		10	27.53±0.25^{a}	19.49	63.05±0.24^{e}	2.07
ANOVA			F computed = 810.05 P<0.05		F computed = 320.51 P<0.05	
1		Control	34.20±0.18^{f}	Nil	64.38±0.16^{e}	Nil
2		2	30.45±0.14^{e}	10.96	60.78±0.25^{a}	5.59
3	Molasses	4	29.17±0.16^{d}	14.72	62.05±0.22^{b}	3.62
4		6	28.62±0.12^{c}	16.33	62.57±0.12^{c}	2.82
5		8	28.20±0.26^{b}	17.54	62.93±0.33^{d}	2.25
6		10	27.93±0.23^{a}	18.32	63.18±0.16^{d}	1.86
ANOVA			F computed = 929.24 P<0.05		F computed = 179.68 P<0.05	

Means with similar superscript in columns are non-significant (P>0.05) with each other. Duncan's multiple range test with level of significance = 0.05.

3.4. Effect of Media and Media Concentration on Biodelignification

Table 3 shows that maximum lignin content reduction compared to control was observed at 4% media dosage using any of the media either molasses or malt extract broth. For malt extract broth lignin content was noted to be 28.85% with a calculated loss of 15.64% respectively. Whereas in case of molasses lignin content was found to be 29.17% with a calculated loss percent of 14.72%. Therefore according to the calculation, lignin degradation using malt extract broth is 0.92% more than molasses at optimum conditions. If holocellulose yield is considered the loss percent is 0.54% more with malt extract broth than molasses. ANOVA showed significant difference between lignin and holocellulose degradation by varying media concentration of malt extract broth and molasses. On the basis of above observations more delignification was found with malt extract broth but if we consider the commercial aspect the molasses are economically 10 times cheaper compared to malt extract broth and saves holocellulose yield. In this view molasses was used to conduct further experiments.

Table 4: Effect of pH on Lignin and Holocellulose Degradation by Schizophyllum commune

S. No.	pH	Lignin (%)	Lignin Loss (%)	Holocellulose (%)	Holocellulose Loss (%)
1	Control	34.20±0.18^{e}	Nil	64.38±0.16^{f}	Nil
2	4.5	31.25±0.10^{d}	8.63	62.92±0.28^{e}	2.27
3	5	30.28±0.12^{c}	11.45	62.50±0.17^{d}	2.92
4	5.5	29.70±0.22^{b}	13.16	62.27±0.19^{cd}	3.28
5	6	29.17±0.16^{a}	14.72	62.05±0.23^{bc}	3.62
6	6.5	29.58±0.12^{b}	13.50	61.87±0.33^{ab}	3.90
7	7	30.20±0.13^{c}	11.70	61.63±0.16^{a}	4.27
ANOVA		F computed = 743.95 P<0.05		F computed = 100.17 P<0.05	

Means with similar superscript in columns are non-significant (P>0.05) with each other. Duncan's multiple range test with level of significance = 0.05.

3.5. Effect of pH on Biodelignification

Table 4 shows that more lignin content reduction compare to control is observed at pH 6.0. The lignin content was 29.17% with a loss percent of 14.72%. If holocellulose yield is considered, the loss percent comes to be only 3.62%. Therefore, according to the analysis pH 6.0 was considered as optimum and used as initial pH for all further studies.

Table 5: Effect of Temperature on Lignin and Holocellulose Degradation by Schizophyllum commune

S. No.	Temperature (°C)	Lignin (%)	Lignin Loss (%)	Holocellulose (%)	Holocellulose Loss (%)
1	Control	34.20±0.18^{e}	Nil	64.38±0.16^{e}	Nil
2	20	33.00±0.19^{d}	3.51	64.02±0.20^{d}	0.56
3	25	29.17±0.31^{a}	14.72	62.05±0.23^{a}	3.62
4	30	29.78±0.15^{b}	12.91	62.60±0.17^{b}	2.76
5	35	31.40±0.11^{c}	8.19	63.62±0.12^{c}	1.19
ANOVA		F computed = 623.31 P<0.05		F computed = 141.44 P<0.05	

Means with similar superscript in columns are non-significant (P>0.05) with each other. Duncan's multiple range test with level of significance = 0.05

3.6. Effect of Temperature on Biodelignification

Lignin percent was less in the sample kept at 25^{0}C. The lignin loss percent calculated was 14.72%. If holocellulose yield is considered, the loss percent comes to be only 3.62%. Thus the optimum temperature for best results was 25^{0}C (Table 5).

4. CONCLUSION

This work put in evidence the importance of fungal pre-treatment in biopulping processes. Work has demonstrated the ability of *Schizophyllum commune* to penetrate the dense chips. The extent of biodelignification clearly shows the advantage of fungal pre-treatment in biopulping processes. According to the observations, the optimum conditions noted for the treatment were: 4% media dose i.e. molasses with initial pH 6.0. Moisture content was kept as 60%. The best results were

obtained at 25^0C temperature and the optimum time for degradation was found to be 28 days in the samples. The treated samples have shown higher lignin degradation at all optimum conditions. In view of this treated samples had shown 14.72% biodelignification at the optimum parameters.

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