Studies on Biopulping of Jute Stick using Phanerochaete chrysosporium

SK Masud Hossain[a], Manas Das[b], S H Ibrahim[c] and N Anantharaman[d]

[a-c]Department of Chemical Engineering, Mohamed Sathak Engineering College Kilakarai 623 806, [b]Department of Chemical Engineering, University of Calcutta, 92, AP.C. Road, Calcutta 700009.

[d]Department of Chemical Engineering, National Institute of Technology, Tiruchirapalli 620 015.

In the present investigations, the effective uses of *Phanerochacte chrysosporium* for biopulping (biodegradation of lignin from raw material) is analysed. The white - rot fungi *P. chrysospurium* (MTCC-787) can degrade lignin from pretreated lingo cellulosic agro-residue jute stick. The optimum inoculation time is 20 days with pH 4.5 and temperature is 35 °C that can delignify maximum percentage. 7 days age – old with 30% (v/v percent of total water) inoculum suspension degrades major percentage of lignin which are optimum parameters. 2.0% (w/w percent of Jute stick) glucose and 0.20 percent (w/w percent of Jute stick) L-alanine (organic nitrogen source) degrades lignin maximum 74.27% of Jute stick with minimum kappa value (16.30). The optical and mechanical properties of pulp obtained by using optimum parameters are very satisfactory and promising for pulp and paper industry. The effluents contain least polluting parameters such as COD, BOD and colour (OD at 465nm).

INTRODUCTION

By drawing an analogy between the metabolism of lignin related aromatic compounds, Kirk et al [1,8], and Eriksson[5] reported that the most important microbe is *Phanerochacte chrysosporium in* lignin biodegradation. The use of white - rot fungi *P. chrysosporium* has predominantly been recommended for the degradation of lignin. Kirk and Yang [2] demonstrated that other basidiomycetes gave similar results, although *Phanerochacte chrysosporium* was most rapid. The idea of using lignin degrading fungus and enzymes for pulping is referred to as biopulping.

The present aerobic batch investigation was undertaken to develop an effective microbial process for login biodegradation using white - rot fungi *Phanerochaete chrysosporium*. In our present works, attempts have been made to optimize process parameters like digestion time, age and concentration of inoculum, pH, temperature, glucose and L-alanine percent (w/w percent of raw material) for biopluping of pretreated lignocellulosic agro - residue jute stick using suspended aerobic grown batch culture of the fungus. The pollution parameters like COD, BOD and colour (OD at 465nm) were also measured for effluents.

EXPERIMENTAL

Collection of microbe

The freeze- dried microbe *Phanerochacte chrysosporium* (MTCC - 787) was collected from Institute of Microbial technology, Chandigarh. The slant cultures were prepared with prescribed growth medium for a period of seven days incubation at 37°C temperature. The microbe was subcultured regularly within 30 days and stored in freeze.

Table-1

Physico - chemical analysis of jute stick (oven dry basis in wt %).

Particular	Amount in percentage
Ash	0.79
Lignin(Klason)	24.69
α- Cellulose	47.86
β- Cellulose	2.42
γ- Cellulose	Nil
Hemicellulose	18.65
Degree of polymerization (viscosity method)	898
Remaining are extractives, etc.	

Table- 2

Analysis of chemically treated raw material.

Characteristics	Amount (%)
Ash	0.32
Lignin(Klason)	10.30
α- Cellulose	60.25
β- Cellulose	3.30
γ- Cellulose	Nil
Hemicellulose	12.96
Lignin degradation	58.25
Kappa number	30.65
Degree of polymerization	875
Yield loss	20.45

Table-3

Optical and mechanical properties of biopulp.Analysis of chemically treated raw material.

Characteristics	Magnitudes
Tear Index, ($mN.m^2/g$)	4.35
Burst Index, ($KPa.m^2/g$)	3.41
Tensile Index, (Nm/g)	32.75
Breaking length (km)	4.83
Brightness (% Absolute)	41.25

Collection of raw material

The raw material jute stick was collected from Tamilnadu Agricultural University, Coimbatore and stored in laboratory at room temperature and atmospheric pressure. 25 mesh size was taken for all experiments. The Jute stick were analysed for various physico-chemical characteristics (Table 1).

Pretreatment of raw material

The pretreatment of raw material jute stick was done with total alkali was 15g Na_2O / lit and sulphidity was 25 percent. Liquor - raw material ratio was 5g liquor/g raw material. The cooking time was 2 h at temperature 95° C by indirect heating with steam in an autoclave system. It was washed with distilled water followed by analysis (Tables 2&3).

Effect of time

The batch process of biopulping (biodegradation of lignin) were operated in 5L Erlenmeyer flasks (digester) containing 40g pretreated raw material. Measured amount of sterilized distilled water was added to wet

Table -4 Effect of time on biodegradation of lignin

Characteristics	Amount						
	BP^4	BP_8	BP_{12}	BP_{16}	BP_{20} *	BP_{24}	BP_{28}
Lignin(Klason)	9.05	7.55	6.10	4.85	3.05 (9.90)	3.05	3.05
α- Cellulose	62.75	65.60	67.40	70.10	74.65 (61.30)	74.85	74.85
β- Cellulose	3.53	3.82	4.16	4.64	5.75 (3.49)	5.79	5.86
γ- Cellulose	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Lignin degradation	12.14	26.70	40.78	52.90	70.25 (3.90)	70.25	70.25
Kappa number	28.10	24.35	21.40	19.85	18.05 (29.35)	18.05	18.05
Degree of polymerization (viscosity method)	872	861	850	843	843 (875)	831	830
Yield loss	NOT DETERMINED			27.62	(5.45)		

* Optimum digestion time

Remainings are ash, hemi- Cellulose, extractives, etc.

BP_4 : Bio pulp after 4 days digestion (innoclation).

BP_8,BP_{12},BP_{16},BP_{20},BP_{24},BP_{28} : Biopulp after 8,12,16,20,24 & 28 days of digestion (inoculation) respectively.

Moisture content during digestion is 245.0 percent.

Blank Experiments (without fungi) values are tabled in ( ).

Table -5 Effect of pH on biodegradation of lignin

Characteristics	pH value			
	3.5	4.5*	5.5	6.5
Lignin(Klason)	3.45	2.95	3.65	4.80
	(9.90)	(9.90)	(9.90)	(9.90)
α- Cellulose	70.25	76.80	71.20	66.35
	(61.30)	(61.30)	(61.30)	(61.30)
β- Cellulose	5.85	5.98	5.41	4.86
	(3.47)	(3.49)	(3.51)	(3.56)
γ- Cellulose	Nil	Nil	Nil	Nil
Lignin degradation	66.50	71.35	64.56	53.40
	(3.90)	(3.90)	(3.90)	(3.90)
Kappa number	18.20	17.60	18.85	19.75
	(29.35)	(29.35)	(29.35)	(29.35)
Degree of	802	843	812	810
polymerization	(862)	(875)	(865)	(848)
(viscosity method)				
Yield loss	33.75	27.62	29.35	31.50
	(5.45)	(5.45)	(5.45)	(5.45)

Analysis after 20 days of digestion.
*Optimum pH value
Remaining are ash, hemicelluloses , extractives, etc.
Moisture content during digestion is 265.0 percent.
Blank Experiments (without fungi) values are tabled in ( ).

Table-6 Effect of temperature on biodegradation of lignin

Characteristics	Temperature, °C			
	30	35 *	40	45
Lignin(Klason)	3.15	2.90	3.30	4.60
	(9.90)	(9.90)	(9.90)	(9.90)
α- Cellulose	74.20	76.95	71.15	68.40
	(61.30)	(61.30)	(61.30)	(61.30)
β- Cellulose	5.61	5.85	5.65	5.62
	(3.43)	(3.49)	(3.61)	(3.61)
γ- Cellulose	Nil	Nil	Nil	Nil
Lignin degradation	69.42	71.84	67.96	55.34
	(3.90)	(3.90)	(3.90)	(3.90)
Kappa number	17.90	17.45	18.00	19.65
	(29.35)	(29.35)	(29.35)	(29.35)
Degree of	809	815	806	796
polymerization	(855)	(875)	(855)	(859)
(viscosity method)				
Yield loss	28.50	27.62	30.20	32.48
	(5.45)	(5.45)	(5.45)	(5.45)

Analysis after 20 days of digestion.
*Optimum temperature
Remainings are ash, hemicelluloses , extractives, etc.
Moisture content during digestion is 260 percent.
Blank Experiments (without fungi) values are tabled in ( ).

the material. 20% (v/v) of inoculum suspension grown aerobically and batch cultured for 7 days at 37 °C temperature in culture medium was transferred aseptically into the reactors. Fungal count was (5-6) x 10^8 numbers per ml in the inoculums suspension (Luckey Drop Method). 500 ml sterilized culture media and 3% glucose (calculated on raw material) and 0.3% Lalanine were added to the reactors. The pH of the reactor was adjusted to 4.5 .The aerobic condition in the system was maintained by putting non absorbent cotton to the month of the rectors. The constant temperature of the reactors was maintained at 35 °C in a constant temperature water bath with constant shaking (rpm was 20) during the digestion. The reactors were transferred after 4,8,12,16,20,24 and 28 days of innoculation respectively. The pulps obtained after specific digestion time called biopulp were thoroughly washed with distilled water followed by analysis (Table 4). The pulp is obtained after biodegradation of lignin with fungi (ie. biopulping) is called biopulp.

Effect of pH

The experimental procedures as stated in effect of time were performed accordingly using pH 3.5,4.5,5.5 and 6.5 . After 20 days of inoculation (optimum digestion time), biopulps were analysed for various physicochemical properties (Table 5).

Effect of temperature

The experimental procedures as stated in effect of time were performed accordingly by adjusting temperatures 30, 35, 40 and 45 °C respectively. After 20 days of inoculation bio pulps were analyzed (Table 6).

Effect of inoculum suspension age

The above said experimental procedures were performed accordingly with 3, 5, 7 and 9 days age - old inoculum suspension. After 20 days of inoculation(optimum digestion time), biopulps were analysed (Table 7) for physicochemical process.

Effect of inoculum suspension concentration

The experimental procedures stated in effect of time was performed accordingly with 10,20,30 and 40% (v/v added water) innoculums suspension of 7 days (optimum) age old culture. Bio pulps were analyzed after

Table -7 Effect of inoculum age on biodegradation of lignin

Characteristics	Inoculums age (days)			
	3	5	7*	9
Lignin(Klason)	3.90	3.45	2.90	4.30
	(9.90)	(9.90)	(9.90)	(9.90)
α- Cellulose	70.15	73.45	77.20	74.60
	(61.30)	(61.30)	(61.30)	(61.30)
β-Cellulose	5.42	5.66	5.83	5.79
	(3.61)	(3.61)	(3.61)	(3.61)
γ- Cellulose	Nil	Nil	Nil	Nil
Lignin degradation	62.14	66.50	71.84	58.25
	(3.90)	(3.90)	(3.90)	(3.90)
Kappa number	19.15	18.20	17.45	19.50
	(29.35)	(29.35)	(29.35)	(29.35)
Degree of polymerization (viscosity method)	802	846	810	781
	(875)	(875)	(875)	(875)
Yield loss	27.62	27.62	27.62	27.62
	(5.45)	(5.45)	(5.45)	(5.45)

Analysis after 20 days of digestion (oven dry basis). All are average values.

*Optimum age of inoculums suspension

Remaining are ash, hemicelluloses , extractives, etc.

Moisture content during digestion is 275.0 percent.

Blank Experiments (without fungi) values are tabled in ( ).

Table - 8 Effect of inoculums concentration on biodegradation of lignin

Characteristics	Innoculum concentration (v/v %)			
	10	20	30*	40
Lignin(Klason)	3.85	3.40	2.90	4.55
	(9.90)	(9.90)	(9.90)	(9.90)
α- Cellulose	71.60	74.25	77.20	73.65
	(61.30)	(61.30)	(61.30)	(61.30)
β- Cellulose	4.51	4.74	5.92	6.78
	(3.61)	(3.61)	(3.61)	(3.61)
γ- Cellulose	Nil	Nil	Nil	Nil
Lignin degradation	62.62	67.00	71.84	55.83
	(3.90)	(3.90)	(3.90)	(3.90)
Kappa number	19.00	18.10	17.45	19.55
	(29.35)	(29.35)	(29.35)	(29.35)
Degree of polymerization (viscosity method)	796 (875)	791 (875)	802 (875)	816 (875)

Analysis after 20 days of digestion (oven dry basis) .
*Optimum innoculum concentration
Remaining are ash, hemicelluloses , extractives, etc.
Moisture content during digestion is 275.0 percent.
Blank Experiments (without fungi) values are tabled in ( ).

20 days of inoculation for physico chemical properties (Table 8).

Effect of glucose and L-alanine dose

The experimental procedures as stated in effect of time were conducted accordingly with various GNC doses ie. Glucose - Nitrogen Combination Doses (GNC dose I, GNC dose II, GNC dose III, GNC dose IV and GNC dose V) as stated with Table - 9. 7 days age old and 30 percent (v/v) which are optimum parameters of inoculums suspension were used for the experiment. Glucose and L-alanine are used as the carbon and organic nitrogen sources to the microbe respectively. The biopulps were analyzed for physics chemical properties after 20 days of inoculation (Table 9). The effluents (filtrate) were also analyzed(Table 10). The optical and mechanical properties of biopulps were shown in Table 11.

Culture media preparation

The following constituents were used for culture media preparation per litre.

KH_2PO_4 .20g ,$MgS0_4$ 5g, $CaCl_2$ 1g, $CuSO_4$ 0.1g ,$ZnSO_4$.$7H_2O$ 0.1g $CuSO_4$ 0.1g, Alk(SO_4) .12 H_2O 0.0lg, H_3PO_3 0.0lg, Na_2MoO4. $2H_20$ 0.0lg, Glucose 10g ,Ca-oxalate 1.5g ,veratryl alcohol 10 percent, 2,2 - dimethyl succinate (0.lM) 10 percent ,L-alanine 0.01 percent (w/v).

Blanck experiments for all parameters

Blanck experiments were conducted for above all said process parameters without microbe Phanerochacte chrysosporium maintaining all the conditions. The results are given in brackets in all tables.

RESULTS AND DISCUSSION

Analysis of raw material and pretreated raw material

The physico - chemical analysis of Jute stick is shown in Table 1. The lignin percentage is 24.69. Total cellulose is 50.22 % with degree of polymerization of 896 (viscosity method). The percentage of hemicellulose 18.65 in jute stick. The characteristic of Jute stick is influenced by factors such as soil, irrigation water quality, amount of water applied, species of Jute cultivated, seasonal rain fall etc. Jute stick has been used for commercial pulping and is considered to be one of the solutions for raw material shortage. The use of jute stick for pulp and paper production is increasing wide spread. Technology of the process has advanced rapidly and several grades of paper are now made, including fine glades. The α - cellulose portion of the jute stick fibre is a potential source of dissolving pulp for the textile and rayon, industry.

Table 2 shows the analysis of chemically treated raw material, jute stick. The pulp obtained by this process has been used for microbial treatment. The lignin degradation is 58.25% with kappa value 30.65. The cellulose yield is 60.25% with degree of polymerization of 875 (Table 2). Hemicellulose presence is 12.9%. The

Table - 9 Effect of glucose and L- alanine dose on biodegradation of lignin

Characteristics	GNC Dose				
	I	II	111*	IV	V
Lignin (Klason)	3.40	2.95	2.65	3.10	3.95
	(9.90)	(9.90)	(9.90)	(9.90)	(9.90)
α- Cellulose	74.60	76.40	80.65	75.35	72.35
	(61.30)	(61.30)	(61.30)	(61.30)	(61.30)
β- Cellulose	5.68	6.95	7.06	6.21	6.61
	(4.50)	(4.50)	(4.50)	(4.50)	(4.50)
γ- Cellulose	Nil	Nil	Nil	Nil	Nil
Lignin degradation	67.00	71.35	74.27	69.90	61.65
	(3.90)	(3.90)	(3.90)	(3.90)	(3.90)
Kappa number	18.10	17.60	16.30	18.05	19.25
	(29.35)	(29.35)	(29.35)	(29.35)	(29.35)
Degree of polymerIzation (viscosity method)	802 (850)	821 (860)	815 (875)	803 (875)	804 (860)
Hemi cellulose	9.32	9.61	9.95	9.87	9.56
	(12.36)	(12.36)	(12.36)	(12.36)	(12.36)
Yield loss	26.10	25.79	24.36	27.62	26.50
	(5.45)	(5.45)	(5.45)	(5.45)	(5.45)

*Optimum GNC Dose
Remaining are ash, hemicelluloses , extractives, etc.
GNC Dose: Glucose - Nitrogen Combination Dose
GNC Dose - I : Glucose 0.5%+ and L - alanine 0.05%+
GNC Dose - II: Glucose 1.0% and L - alanine 0.10%
GNC Dose - III: Glucose 2.0% and L - alanine 0.20%
GNC Dose - IV: Glucose 3.0% and L - alanine 0.30%
GNC Dose - V : Glucose 4.0% and L - alanine 0.40%
GNC Dose - VI : Glucose 5.0% and L - alanine 0.50%
+ Percent meant w/w percent of jute stick.
Moisture content during digestion is 275.0 percent.
Blank Experiments (without fungi) values are tabled in ( ).

physical and mechanical properties of chemically treated pulp is shown in Table 3.

Effect of time

The effect of time on optimum lignin degradation of pretreated Jute stick with basidiomycetes *Phanerochaete chrysosporium* is shown in Table 3. Microbial degradation of lignin using *P. chysosporium* occurs after three days of inoculation after which lignin mineralized to carbon dioxide. Therefore in your studies, properties of biopulp were analyzed after 4 days of digestion. This time is required by the fungus for the full growth and adaptation by the fungus on the solid substrate system. The fungus gas grown over the solid substrate, depleted the available nitrogen, and become ligninolytic. This period is also avoidance of lag-phase. The microbial process often utilizes the activities of the adaptive enzymes of the microorganisms. The depletion of limiting nutrient nitrogen triggers the development of ligninolytic system and is therefore necessary for biodegradation of lignin.

It is observed that optimum digestion time is 20 days. The lignin degradation is 70.25% with kappa number of 18.05 achieved after 20 days of innoculation (Table 4). After this, the delignification rate gradually slows down. This can be explained on the basis of microbial activity phenomena and nutrients. The enzyme activity of the fungus slows down.

The limiting nutrients levels for the effective activity of

Table-10 Analysis of effluents

Characteristics	GNC Dose				
	I	II	III*	IV	V
COD,mg/l	1,585	1,450	1,275	1,460	1,485
	(2,690)	(2,690)	(2,740)	(2,785)	(2,930)
BODs, mg/l	890	865	850	875	915
	(1,405)	(1,450)	(1,450)	(1,475)	(1,490)
Colour	0.34	0.34	0.32	0.32	0.35
(OD at 465 nm)	(0.45)	(0.45)	(0.46)	(0.46)	(0.45)

Filtrate after 20 days of digestion with various GNC doses

* Optimum GNC Dose

Blank Experiments (without fungi) values are tabled in parenthesis.

Table-11 Optical and mechanical properties of biopulp.

Characteristics	GNC Dose				
	I	II	III*	IV	V
Tear Index,	6.35	6.60	7.85	6.85	5.90
($mN.m^2/g$)	(4.50)	(4.50)	(4.50)	(4.50)	(4.50)
Burst Index,	5.60	5.85	6.34	5.55	5.15
($KPa.m^2/g$)	(3.55)	(3.55)	(3.55)	(3.55)	(3.55)
Tensile Index,	50.65	53.40	55.42	51.60	50.30
(Nm/g)	(33.05)	(33.05)	(33.05)	(33.05)	(33.05)
Breaking length (km)	6.60	6.75	7.28	6.65	6.25
	(4.83)	(4.83)	(4.83)	(4.83)	(4.83)
Brightness (or)	66.80	68.35	70.60	67.20	62.15
(% Absolute)	(41.90)	(41.90)	(41.90)	(41.90)	(41.90)

Analysis after 20 days of digestion with various GNC doses (oven dry basis).

* Optimum GNC Dose

Blank Experiments (without fungi) and chemically treated values are tabled in parenthesis.

microbes decreases to such an extent that the fungal function is deactivated. The cellulose yield is 74.65% with degree of polymerization (DP) 843 (viscosity method) after 20 days incubation. The biodegradation process being dependent on the extra cellular lignin peroxides enzyme secretion. The enzymes ligninase and manganenase necessary for lignin biodegradation has been responsible to be regulated by the concentrations stimulating the production of the lignin degrading enzymes (1-12). Thus for our further studies for various parameter optimization, 20 days is taken as optimum inoculation time.

Effect of pH

The effect of pH on biodegradation of lignin with P. Chrysosporium is shown in Table 4. The range of pH 3.5 - 6.5 is experimented. It is shown (Table 5) that the lignin biodegradation is maximum 71.35 when pH is 4.5. The cellulose yield is 76.80% with degree of polymerization(DP) is 843. The least kappa value is 17.60 at pH 4.5(Table 5). Therefore, pH 4.5 is the optimum for delignification with Phenerochaete Chrysosporium. When pH is lower or higher to 4.5, the yield of cellulose is lower along with lower delignification. Therefore kappa value is higher (Table 5).

Effect of temperature

Table 5 shows the effect of temperature on biodegradation of lignin with microbe. The range of temperature 30 - 45°C is experimented. Lignin degradation is maximum

(71.84) when the temperature is 35°C. The cellulose yield is 76.95% with degree of polymerization (DP) 815 (viscosity method). The kappa number value is 17.45 for 35°C (Table 6). Hence the optimum temperature is 35°C for maximum delignification of jute stick. When temperature increases (above 35°C), the delignification declines which results in declination of cellulose yield and higher kappa number (Table 6).

Effect of inoculum age

The effect of inoculums age on lignin biodegradation is shown in Table 7. There is an increasing trend of lignin degradation and yield of cellulose up to 7 days age - old of inoculums suspension. 7 days age - old inoculum suspension favours the maximum (71.84) percentage of lignin degradation with lowest (17.45) kappa value. The cellulose yield is 77.20% (maximum) with degree of polymerization is 810 for 7 days age-old inoculum suspension (Table 7). 5 days age-old and 9 days age-old inoculum suspension can degrade lignin upto 66.50 percent and 58.25% respectively. 7 days age-old inoculums suspension favors maximum delignification of pretreated jute stick after 20 days of inoculation (Table 7).

Effect of innoculum suspension concentration

Table 8 shows the effect of innoculum suspension concentration on physicochemical properties of biopulp. The delignification and cellulose yield have been shown to be proportional to the innoculum concentration. 30% (v/v percent of total added water) favours the maximum (71.84) percentage of delignification with least (17.45) kappa number. The cellulose yield is highest (77.20) percent with degree of polymerization (DP) is 816 (viscosity method). Highest percentage of lignin degradation with least values of kappa number and highest percentage of celluloses yield are the desirable criteria of fungal treatment as well as pulp and paper. The cellulose degradation should be least as possible ie. the degree of polymerization (DP) should be as maximum possible. 40% (v/v of total added water) inoculum concentration degrades lower amount of lignin with lower amount of cellulose comparing 30% inoculum concentration (Table 8). Mechanical forces can disturb the elaborate structure of the enzymes secreted by the fungus to such a degree that deactivation can occur. The forces associated with liquid film and interfaces can all cause deactivation of enzymes. The rate of denaturation is a function both the intensity and time expose flow regime. Another mechanical force higher surface tension, often causes denaturation of proteins and consequent deactivation of enzymes. In this processing context, a combination of mechanical forces and chemical reactions (redox, etc.) deactivates enzymes. Thus higher percentage of innoculum concentration degrades less percentage of lignin with higher Kappa value (Table 8). Thus 30% innoculums suspension is the optimum dose for maximum biodegradation of lignin.

Effect of glucose and L-alanine dose

The purpose of adding lingo - cellulosic with glucose is two-fold. Firstly, addition of glucose promotes the growth and establishment of the fungus within the lingo-cellulose materials. Secondly, white- rot fungi Phanerochate chrysosporium needs an additional easily metabolized carbon source to degrade lignin. Thus if the lignocelluloses are mixed with glucose, the celluloseless mutant would be preferentially metabolize the added glucose and naturally occurring low molecular mass sugars instead of degrading the cell wall hemicelluloses during the delignification thereby protecting the fibres' [2,8,9& 11] . Thus if the lingo - celluloses are glucose -amended, the highest radio of lignin loss to cellulose loss will occur. In absence of glucose, there is more fungal attack of pulp rendering the fibres more suspectable to mechanical action [8,9,& 15] . Nitrogen metabolism plays a role in regulating lignin degradation as a part secondary metabolism in the basidiomycete *Phanerochate chrysosporium*. Other predetermined aspects of the choice of nitrogen source can also influence the choice of nitrogen source and concentration. Lignolysis being strongly inhibited by high nitrogen concentration. Lignin degradation begins only after the cessation of linear growth possibly in response to N-starvation [15 & 23].

For optimization studies of glucose and L-alanine (as organic nitrogen source) ,a wide range of glucose concentration (w/w percent of Jute stick) from 0.5 to 5 percent and L-alanine from 0.05 to 0.50 percent (w/w percent of Jute stick) are experimented. Various GNC doses ie. Glucose - Nitrogen Combination Doses viz. GNC dose I ,GNC Doses II , GNC Doses III , GNC Doses IV, GNC Doses V and GNC Doses VI as described with Table- 9, are taken. In case of biodegradation of lignin with *Phanerochate chrysosporium*, lignin degradation with microbe increase with the increase in GNC Dose up to GNC Dose III and any further increase of glucose - nitrogen concentration, there is sharp decline in lignin degradation (Table 9). At very high glucose and L-alanine concentration (beyond GNC Dose III), deviation from the classical Michaels - Menten growth rate are observed. Since the ligninolytic enzymes are exo enzymes and are regulated by the catabolic repression in the presences of higher concentration of readily metabolized sugar source of glucose, the fungus enzyme

synthesizing machinery switches over to another sugar metabolism in the presence of lignin or its fragments.

GNC Dose - I (0.5% glucose and 0.05% L- alanine) degrades lignin up to 67.00% with kappa number 18.10.GNC Dose - II (0.1% glucose and 0.10% of L-alanine) can degrade lignin up to 71.35% with kappa number value 17.60. Lignin biodegradation with GNC Dose III (2.0% glucose and 0.20% L-alanine) is maximum 74.27% with least value of kappa number (16.30). The yield of celluloses is 80.65 percent with degree of polymerization (DP) is 815 (viscosity method). GNC Dose - IV (3.0% of glucoses 0.30 percent of L- alanine) degrades lignin up to 69.90% with kappa number value is 18.05 (Table 9) . GNC Dose - V (4.0% glucose and 0.40% L-alanine) degree lignin up to 61.65%. GNC Dose - III is the optimum lignin degradation with the microbe (Table-9). Other GNC Doses degrade to some less extend comparing to GNC Dose - III . Higher amount of glucose and' L-alanine are not feasible for economical purpose. Highest percentage of lignin degradation with lowest value of kappa number are the desirable criteria for the good quality pulp and paper as well as bio pulping process. Highest percentage of degree of polymerization (DP) is also the desirable properties of good quality pulp as well as microbial biodegradation of lignin.

From Table-9, it is shown that the delignification can be carried out to 74.27% for GNC Dose - III. This is because of the physical and chemical hindrance owing to the morphological structure of jute stick and existence of fibre matrix among celluloses, hemicelluloses and lignin. This can be attributed to that the presence of proper amount of glucose and L -alanine enhances the clevage linkages between lignin and hemi cellulose. Thus enhances the biodegradation of lignin. Thereby, achieving the stabilization of cellulose chains and higher degree of polymerization. Therefore ,the produced pulp obtained from GNC Dose - III having properties better than the pulp obtained from other GNC Doses (Table 11). This may be due to the fact the surface tension property of the innoculum reduced to such a point which helps in the penetration of micro-organisms into the fibre matrix and diffusion of the breakdown products of lignin into the medium, assuming a homogeneous state of microbes through out the digestors.

The mechanical and optical properties of the biopulps obtained from various GNC Doses treated are shown in Table 11. The mechanical and optical properties of GNC Dose- III treated biopulps are maximum. Maximum brightness is 70.60% for GNC Dose -III treated biopulps. Maximum tear index (7.85mN.m^2/g), maximum burst index (6.34 KPa.m^2/g), maximum tensile index (55.42Nm/g) and maximum breaking length (7.28Km) respectively obtained for GNC Dose-III treated biopulps. The mechanical properties depend on the lignin behaviour that is of importance in pulp quality in the nature and location of residual lignin in fibre. Mechanical and optical properties increase with in the delignification and stabilization of cellulosic chain. The definite amount of glucose and L - alanine accelerates the biodegradation rate and the fibre protection. The tensile property is influenced by degree of fibre collapse and fibre length distribution. Anderson and Mohilin (9) depicted that a decrease in long fiber content was observed for fungus - treated samples without glucose - impregnated. Where as in samples with glucose no such decrease was observed. The glucose impregnation has a beneficial effect on the ability of the fibres to bind each other in the paper sheet, which could be due to a repression of hemicelluloses degradation.

The effluent characteristics of biopulping process is shown in (Table 10). Besides satisfactory lignin degradation, the innoculated fungi *Phanerochacte chrysosporium* is very suitable for pollution - abatement of the effluents(17 -21). GNC Dose-III treated effluent (filtrate) shows the minimum COD ,BOD and colour (OD at 465mm) . COD is 1,175 mg/l, BOD is 850 mg/l and colour is 0.320 (OD at 465mm) which are minimum values for GNC Dose III treated effluents (Table 10) . Other GNC Doses treated effluents (filtrates) show higher values of COD, BOD and colour(Table 10).

Blank experiments (without fungi)

All blank experiments (without fungi) maintaining all the parameters not changeable is shown in ( ) in all tables -3, 4, 5, 6, 7, 8, 9 & 10. The lignin degradation varies from 2-4% (w/w of initial lignin). This can be explained on the basis of constituents present in the culture media, and temperature. The culture medium formulation is an essential stage in the design of successful laboratory experiments and manufacturing processes. The constitutents of the medium satisfies the essential requirements for cell biomass and metabolism and there must be adequate supply of energy for cell maintenance. Besides meeting requirements for growth and product formation, the medium may also influence the oxidation-reduction potential and the morphological form from the organism. It should be possible to calculate the minimal quantities of nutrients which will be needed, to produce a specific amount of biomass. Knowing that a certain amount of biomass is necessary to produce a defined amount of product. Cooney (23) showed that some nutrients are frequently added in substantial excess of that required; however, others are often near limiting values. In specific processes

the concentration of certain minerals may be very critical. The concentrations of Mg, Fe, and Zn are the most critical in secondary metabolism. Weinberg (24) has reported that in every secondary metabolism system, the yield of the product varies linearly with the logarithmic concentration of the "Key" metal. Nutritional factors can after the oxygen demand of the culture. Oxygen is never the less a very important component of bio pulping and its availability can be extremely important in controlling, grown rate, metabolism, media preparation having very very less impact on lignin degradation without fungi.

CONCLUSION

The effective uses of white - rot fungus *Phanerochaete chrysosporium* is critically analysed for biopulping of Jute stick. Aerobic batch grown suspension culture of microbe used for the maximum delignification from batch scale studies. Optimum digestion time is 20 days. Optimum pH of digestion medium is 4.5. Optimum temperature of pulping process is 35°C. Optimum age of innoculum suspension is 7 days. Optimum innoculum concentration is 30% (v/v percent of total water). Optimum glucose and nitrogen combination dose is 2.0% (w/w percent of Jute stick) glucose (as carbon source) and 0.20% (w/w percent of Jute stick), L-alanine (as nitrogen source) that can degrade lignin upto 80.65% (w/w). COD,BOD and color at optimum glucose and L-alanine dose are 1,275 mg/L, 850 mg/L and 0.32 (OD at 465 mm) respectively. At optimum glucose and L- alanine dose, tear index is 7.85 mNm^2/g, burst index is 6.34 $KPa.m^2/g$, tensile index is 55.42 Nm/g , breaking length is 7.28 km and brightness is 70.60% absolute respectively. The lag- phase which is nothing but the adaptation phase of the fungus to the ligninolytic system, should be reduced as much as possible and this may be achieved by using a suitable inoculum media. Detailed investigation is needed to establish the most suitable biopulping medium for the lignocellulosic agro- residue Jute Stick.

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