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Original Article

Bio-Bleaching of Unbleached Kraft Pulp (UKP) by Two White Rot Fungi

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Abstract

Pycnoporus cinnabarinus and *Daedaleopsis flavida,* the two white rot fungal strains were considered very useful in the bleaching of unbleached kraft pulp. The reduction of kappa number and improvement of brightness of kraft pulp was evaluated during the solid state and submerged fermentation. *P. cinnabarinus* responsible for reduction of kappa number from 10.0 (10 days) to 8.3 (15 days) and 7.4 (30 days) during solid stated fermentation (SSF) while the brightness of the pulp which was initially 34% improved up to 58% in 30 days of incubation. The viscosity values were primarily 30.5 was reduced to 28.4 in 15 days and 27.1 in 30 days of incubations. The laccase enzyme of *P. cinnabarinus* caused for improvement of brightness from 33-55% and reduction of kappa number 10.2 to 7.3 and viscosity 28.4 to 25.3. The laccase of *D. flavida* was responsible for increase in brightness from 35-59% while reduction of kappa number 10.2 to 6.8 and viscosity 28.4 to 24.5. The influence of Dupont showed the reduction in kappa number from 10.8 to 0.98 and the hemicelluloses content was reduced from 42.7 to 19.8 in the last concentration (30g/100 Kg of pulp). With novozyme the lignin content was reduced from 0.130 to 0.085% in the 25g/100 Kg concentration. The kappa number was 14.4 in control reduced to 12.2 in the last concentration. The viscosity was reduced from 15.8 to 10.2 in final concentration after 60 minutes of incubation time. The energy and wood costs were drastically reduced in biological procedures.

Keywords: Biobleaching; Unbleached Kraft Pulp; White Rot Fungi; Kappa Number; Viscosity; *Pycnoporus cinnabarinus; Daedaleopsis flavida.*

Introduction

The use of white-rot fungi for the biological delignification of wood was perhaps first seriously considered by Lawson and Still (1957) at the West Virginia Pulp and Paper Company (now Westvaco Corporation) [1]. The literature survey carried out on biopulping by pretreatment of wood chips has shown that the studies are confined only up to evaluation of properties of pulp and paper sheets produced from pretreated chips. However, there is no literature available on accessibility on different fungal strain to the fiber in order to ascertain their effectiveness. Prominent obstacles are that the fungi partially damage cellulose. They have not been successfully used because of their slow speed of degradation and cultivation.

Wood is a natural biodegradable and renewable raw material, used in construction and as a feed stock in the paper and wood product industries and in fuel production. Wood is polymeric composite whose biological and technical properties are mainly determined by the chemical composition of the cell wall. Wood cell walls are made up primarily of cellulose, hemicelluloses and lignin The tensil strength of wood fibers is primarily determined by cellulose and hemicelluloses, while lignin mediates adhesion between the fibers. Cellulose is a linear polymer of high molecular weight, exclusively built up by 1-4 glycoside linked molecular of β-D-Glucose. The chains of the more complex hemicelluloses are much shorter than those of cellulose but they usually bear side groups, such as monosaccharide and acetyl groups and in some cases they are branched [2]. Lignin is three dimensional polymer of Phenylproponoid units which are oxidatively Polymerised by a peroxidases or phenol oxidases

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during lignin biosynthesis [3].

In white rot fungi, wood polysaccharides are degraded during the primary metabolism of the fungus, while lignin degradation due to the variety of chemical bonds occurs only during secondary metabolism and yields no net energy gain [4]. During degradation process, the initial attack on lignin is considered to be performed by extracellular laccases, lignin peroxidases and manganese peroxidase [5]. Peroxidase use hydrogen peroxide as co-substrate, while laccase require molecular oxygen as electron acceptor [6] .

These enzymes catalyse the one electron oxidation of lignin related phenolic groups to relatively stable phenoxy radicals. Kirk and Yong (1979) were the first to attempt to bleach the pulp with microorganisms [7]. They observed that *Phanerochaete chryosoprium* and other white rot fungi could cover the kappa number of unbleached soft wood kraft pulp by up to 75%, leading to reduced requirement for chlorine during subsequent chemical bleaching. Screening of several white rot fungi at the pulp and paper research institute of Canada revealed that *Trametes versicolor* could markedly increase the brightness of hard wood craft pulp and noted that the Kappa number was decreased from 12 to 8, and the brightness increased from 34-48%. Due to the importance of lignin as a renewable source for the production of paper, feeds, chemicals and fuels, there has been an increasing research emphasis on the fungal degradation of lignin. Delignification of UKP by white rot fungi has been investigated by many researchers and its significance in paper industries was recorded [8,9] .

Kirk *et al.,* (1978) used *Phanerochaete chryosoprium*for bio bleaching in liquid state fermentation system and reported the kappa number was decreased by 60%, after a subsequent alkali treatment [10]. Paice *et al.,* (1990) used *Trametes versicolor* in liquid state fermentation system and achieved 15 point increase in brightness [11]. Katagiri *et al.,* (1995) noted the same level of cumulative manganese peroxidase production by *Phanerochaete chryosoprium* and *Trametes versicolor* in the solid state and liquid state fermentation systems but observed negligible increase in brightness in the liquid state fermentation system [12]. Fugita *et al.,* (1993) classified that the bio bleaching process can significantly increase the brightness of the pulp and reduce the use of chlorine based chemicals and pollution load of waste liquor [13]. Rivera *et al.,* (2000) attempted to design a solid state bioreactor for application of the process in the industrial scale [14]. The application of the white rot fungi and their enzymes in the wood based industries for the removal of lignin and increase the brightness of the pulp was thoroughly understood [15-17].

Basidiomycetes are unique in their ability to degrade most components of wood due to their ability to synthesize the relevant hydrolytic and oxidative extracellular enzymes. Laccases were used for the depolymerization of lignin; delignify wood pulps and bleaching of kraft pulps; development of new white rot fungal strains for effective treatment of bleaching actually without causing any damage to cellulose fibrils [18-20]. Gates *et al.,* (2011) used lignin peroxidase and laccase systems to detoxify the Kraft pulp mill effluents by removing the toxic chemicals and at the same time reported their role in the reduction costs in paper manufacturing [21].

In view of these interesting observations on the role of white rot fungi in treatment of kraft pulp for paper manufacture a detailed study was undertaken on the reduction of kappa number and improvement of brightness of the Kraft pulp. The study wars oriented to develop strategies for the effective beaching processes by using the fungi or their enzymes to reduce the paper manufacturing cost and also to reduce the pollution loads.

Material and Methods

The unbleached kraft pulp (UKP) of subabul (*Leucaena leucocephala*) was processed and five grams of oven dried pulp was transferred in to nine conical flasks and sterilized. Five ml of spore suspension (105 spores/ml) of two selected fungi viz., *Pyenoporous cinnanabarinus* and *Daedolepsisi flavida* (Figure 1) was poured in to the conical flask and incubated at room temperature. After incubation of 15 and 30 days one gram of the pulp was digested and used for the estimation of pH, brightness (%), kappa number and viscosity (cp). The flask with (UKP) inoculated with spore suspension sorced as control ('o' incubation). The procedure for all parameters were followed as the method suggested in TAPPI (1993).

Kappa number is used as criteria for the lignin content of pulps and is determined as the volume of 0.1 N potassium permanganate (ml) consumed by 1.0 g of moisture free pulp. A portion of the cut piece of pulp that could consume approximately 50 per cent of potassium permanganate solution (0.1%) was weighted out and disintegrated in 500 ml distilled water until free of fibre clots or bundles. The disintegrated suspension was made up to 800 ml. To 100 ml of KMnO4 solution (0.1 N), 100 ml of H_2SO_4 (4 N) was added and cooled to 25°C and immediately added to disintegrated pulp suspension. After 10 min. the reaction was stopped by adding 20 ml of

potassium iodide solution (1 N) and titrated against sodium thiosulphate solution (0.2 N). Starch solution (0.2%) was used as the indicator. A blank titration was carried out in the same manner but without pulp. The kappa number was calculated by the formula.

K= p x f / W

and

P = (b-a) N / 0.1

Where,

K = Kappa number

F = Factor for correction to the 50 per cent permanganate consumption depending on the volume of p (TAPPI, 1993)

W = Weight of moisture free pulp sample used for estimation (g)

P = Amount of 0.1 N permanganate consumed by the sample (ml)

B = Amount of thiosulphate consumed in blank determination (ml)

A = Amount of thiosulphate consumed by sample

N = Normality of thiosulphate

Correction for reaction temperature

$$K= \frac{PF}{w} [0.0+0.013(25-t)]$$

Where, t = actual reaction temperature in degree celsius.

Brightness

Brightness of the UKP was measured at 457 nm in a Perkin Elmer ë3B spectrophotometer equipped with a reflectance sphere.

Viscosity

The viscosity of the UKP was estimated by using Oswald viscometer.

Results

During the studies on the applications of white rote fungi on paper and pulp industries the pulp used was from the plant subabul *(Leucaena leucocephla)*. This Unbleached Kraft pulp (UKP) was analysed for its different parameters which will have its impact on the bleaching and pulping processes. The yield of the pulp from the plant was 32.2% (Table 1). The permanganate number was 12.6 and viscosity was recorded as 30.8 cp. The ash content was 1.66%, while aluminium, calcium oxide and magnesium oxides were 0.261%, 1.150%, 0.148% respectively. The iron content was 466 ppm and pentosans were 16.5%. The AB extractives were 2.59% while lignin was 27.2% and Holo cellulose was 74.2%.

The impact of white rot fungus, *Pycnoporus cinnabarinus* on the digestion of lignin during solid state fermentation (SSF) was analysed in 15 and 30 days of incubation and recorded (Table 2). From the table it was evident that during SSF the pH gradually decreased towards acidic side. The brightness of the pulp was improved and kappa number, the indicator of lignin content was slowly decreased. The important parameter in the pulp industries, the viscosity was not affected much with the growth of the organism. These studies were conducted with a gap of three months each in 2014 and presented. In the month of January 2014 the initial pH 9.4 was reduced to 8.2 in 15 days incubation and 7.5 in 30 days of incubation, while, the brightness of the pulp is initially 34% was improved to 45% (15 days) and 58% (30 days). The kappa number of the pulp was initially 10 was reduced to 8.3 in 15 days and 7.4 in 30 days of incubations. The viscosity values were primarily 30.5 was reduced to 28.4 (15 days) and 27.1 (30 days).

The similar data was calculated during April 2014 and the pH was initially 8.8 changed to 7.5 and 6.6 in 15 and 30 days of incubation time. The brightness was 35 % improved to 47 and 59% after 15 and 30 days of SSF. The kappa number was decreased to 8.9 to 7.5 in 15 days and 6.4 in 30 days of incubation. The viscosity was reduced from 29.6 to 27.3 and 26.3 in 15 days and 30 days respectively. After three months gap again in July 2014 the fungus was inoculated with UKP and analysed the improvement of brightness and decrease of lignin content. The pH which was initially 7.9 were 7.0 in 15 days and 6.2 in 30 days of incubation. The brightness was initially 35 improved to 45% in 15 days and 56% in 30 days of incubation period. The kappa number which was originally 9.0 was reduced to 8.1 and 7.3 in 15 days and 30 days of incubation period. The viscosity was 29.5 and reduced to 28.1 and 27.3 in 15 and 30 days of incubation period respectively. In October 2014 the pH was changed from 9.0 to 8.0 and 7.1 in 15 and 30 days of incubation period. The brightness was initially 35 was improved to 44 and 54% in 15 and 30 days of incubation. The kappa number was initially 10 reduced to 8.5 and 7.6 in 15 and 30 days incubations respectively. The viscosity was 30.6 in initial period reduced to 28.7 in 15 days and 26.1 in 30 days of incubation period.

Similarly the other potential white rot fungus,

Daedaleopsis flavida, was also studied for its effect on the pH, brightness, kappa number and viscosity during SSF and presented in (Table 3). From the table it was noticed that the pH 9.4 reduced to 8.5 and 7.2 in 15 and 30 days of incubation time. The brightness which was originally 34% was improved to 46 in 15 days and 59% in 30 days of incubation period. The kappa number which was 10 originally reduced to 8.5 and 7.3 during 15 and 30 days of incubation period. The viscosity levels were 30.5 reduced to 27.5 and 26.3 in 15 and 30 days of incubation time. Similar trends were continued in the remaining three incubation periods i.e. April, July and October 2014. The pH reduced slightly but maintained its neutrality by the end of 30 days incubation. The brightness percentage reached 60 in April 2014 after 30 days of incubation, which indicated the efficiency of the organism in replacing the oxidizing chemicals chlorine and hydrogen peroxide. The organism was good enough to digest lignin and its maximum kappa number reduction was witnessed again in April 2014, which was initially 8.9 reduced to 7.2 in 15 days and 6.8 in 30 days of incubation period during SSF. Over all, these two organisms are useful for the bio bleaching due to their activities in improvement of brightness of the pulp and decreasing the kappa number while not affecting much the viscosity of the pulp.

To understand the difference between the chemical bleaching and biological bleaching the data with chemical bleaching was also recorded in (Table 4). From the table it was evident that the original brightness of the pulp was 48% gradually improved in different chemical treatment stages with chlorination and hydrogen peroxide and at last reached to 94%. Similarly the kappa number which was originally 10 reduced to 0.5 levels in the last stage of chemical treatment. The viscosity was reduced from 30.5 to 17.5 at the final stage.

The bio bleaching of UKP with laccase enzyme of *P. cinnabarinus* was studied and reported (Table 5). From the table it was evident that the brightness of the pulp was increased from 35-55% with *P. cinnabarinus* while it was from 35-59% by *Daedaleopsis flavida.* The kappa number reduced from 10.2 to 7.3 by *P. cinnabarinus* while it reduced to 6.8 with *Daedaleopsis flavida.* There was a marginal reduction in the viscosity *P. cinnabarinus* which reduced from 28.4 to 25.3 while it was 28.4 to 24.5 with *Daedaleopsis flavida.* The enzyme laccase showed substantial improvement in the pulp treatment which helped for the improvement of brightness and reduction of lignin content (kappa number).

The influence of microbial enzymes commercially available with trade names, Dupont enzyme and Novozyme were analysed on the important pulp properties essential in paper industries were recorded in table 1. The influence of Dupont enzyme in its three concentrations i.e. 10g/100 kg pulp; 20g/100 kg pulp; 25g/100 kg pulp and after 60 minutes incubation the lignin content was reduced from 0.168% to 0.121% in its last concentration (Table 6). The kappa number was initially 10.8 was reduced to 10.2 in 10g/100kg concentration and 0.98 in last concentration (25g/100kg). The reactivity i.e. hemicellulose content was 42.7 in control and reduced gradually to 19.8 in last concentration. The viscosity was not affected with this enzyme and reduced from 14.5 to 9.8. Similarly the influence of Novozyme was also studied and the lignin content reduced from 0.130 to 0.085% in the 25g/100kg concentration (Table 7). The kappa number was 14.4 in control and reduced to 12.2 by the last incubation concentration. The reactivity percentage was 37.4 initially and reduced to 13.4 in 25g/100 kg concentration, while the viscosity was 15.8 in control and 10.2 in last concentration.

Pycnoporus cinnabarinus *Daedaleopsis flavida*

Fig. 1: Showing the fruit bodies of macro fungi

Table 1: Analysis of unbleached kraft pulp (UKP) of subabul (*Leucaena leucocephala*)

Yield (%)	32.2
Permanganate number	12.6
Viscosity(CP)	30.8
Ash (%)	1.66
Al (%)	0.261
Cao (%)	1.150
Mgo (%)	0.148
Iron(PPM)	466
Pentosens (%)	16.5
AB Extractives (%)	2.59
Lignin (%)	27.2
Holo Cellulose (%)	74.2

Table 2: Influence of *Pycnoporus cinnabarinus* on pH, brightness, kappa number and viscosity of UKP during solid state fermentation after 15 and 30 days of incubation

	pH			Brightness (%)			Kappa number			Viscosity (cp)		
	0	15d	30d	0	15d	30d	0	15d	30d	0	15d	30d
Jan. 2014	9.4	8.5	7.5	34	45	58	10.0	8.3	7.4	30.5	28.4	27.1
Apr. 2014	8.8	7.5	6.6	35	47	59	8.9	7.5	6.4	29.6	27.3	26.3
Jul. 2014	7.9	7.0	6.2	35	45	56	9.0	8.1	7.3	29.5	28.1	27.3
Oct. 2014	9.0	8.0	7.1	35	44	54	10.0	8.5	7.6	30.6	38.7	26.1

Table 3: Influence of *Daedaleopsis flavida* on pH, brightness, kappa number and viscosity of UKP during solid state fermentation

	pH			Brightness (%)			Kappa number			Viscosity (cp)		
	0	15d	30d	0	15d	30d	0	15d	30d	0	15d	30d
Jan. 2014	9.4	8.5	7.2	34	45	59	10.0	8.5	7.3	30.5	27.5	26.3
Apr. 2014	8.8	7.3	6.8	35	48	60	8.9	7.2	6.8	29.6	28.3	27.8
Jul. 2014	7.9	6.8	6.0	35	47	58	9.0	8.0	7.1	29.5	28.3	27.5
Oct. 2014	9.0	8.1	7.0	35	45	56	10.0	8.2	7.2	30.6	28.5	26.0

Table 4: Brightness, kappa number and viscosity changes during chemical bleaching of UKP of subabul pulp

Bleaching Stage	Brightness (%)	Kappa number	Viscosity (cp)
Un bleacher kraft pulp	48	10	30.5
Chlorination stage	57	8	28.7
Oxygenated H_2O_2 stage	68	6	25.3
Hypo 1 stage	73	4	22.1
ClO_2	81	3	20.5
Extracted peroxidase stage (EP)	90	2	19.2
Final brightness pulp	94	0.5	17.5

Table 5: Biobleaching of UKP with laccase enzyme of *Pycnoporus cinnabarinus* (A) and *Daedaleopsis flavida* (B) after 30 days of solid state fermentation

Treatment	Brightness (%)		Kappa number		Viscosity (cp)	
	A	B	A	B	A	B
Untreated pulp	35	35	10.2	10.2	28.4	28.4
Laccase treated pulp	55	50	7.3	6.8	25.3	24.5

Table 6: Influence of dupont enzyme on the biobleaching of kraft pulp

Enzyme concentration	Lignin (%)	Kappa Number	Reactivity hemicellulose (%)	Viscosity (cp)
Control	0.168	10.8	42.7	14.5
10g/100 kg	0.140	10.2	35.8	12.9
20g/100 kg	0.135	10.8	29.4	11.8
25g/100 kg	0.121	0.98	19.8	9.8

Table 7: Influence of novozyme on the bio bleaching of kraft pulp

Enzyme concentration	Lignin (%)	Kappa Number	Reactivity hemicellulose (%)	Viscosity (cp)
Control	0.130	14.4	37.4	15.8
10g/100 kg	0.094	13.6	27.4	12.8
20g/100 kg	0.090	12.8	25.4	12.1
25g/100 kg	0.085	12.2	13.4	10.2

Table 8: Comparison of conventional and bio pulping process in manufacturing costs (in Rs./Ton) of paper

Cost	Conventional	Biopulping
Energy	9680	6400
Wood	6720	1280
Bleaching chemicals	800	800
Other costs	4800	4800
Total:	22000	13280

The cost of paper manufacturing in conventional type and bio pulping process was compared and presented (Table 8). From the table it was clear that the energy costs were reduced drastically with bio pulping. The energy cost in conventional method was Rs. 9680 reduced to Rs. 6400 in the biological treatment process. The wood costs during paper manufacturing also showed remarkable reduction. It was Rs. 6720/- per ton in conventional while it was only 1280/- per ton in bio pulping. No change was recorded in the cost of bleaching chemicals and other costs of paper manufacturing.

Bio pulping and Bio bleaching would have a bright and promising future in pulp and paper industry because of its both economizing energy and friendly to environment. Now, it is necessary to enhance this process of lignin bio degradation and bio pulping which is very significant meaning to the sustaining development of pulp and paper industry.

Discussion

White rot fungi are most effective for delignification due to production of lignolytic extracellular oxidative enzymes. Lignin degradation was possible by several white rot fungi, such as *Phanerochaete chrysosporium*, *Pleurotus ostreatus*, *Trametes versicolor*, *Cyathus sterours, Certiporiopsis subvermispora* [22,23] . They noticed that some white rot fungi delignify wood by preferentially attacking lignin more readily than hemicelluloses and cellulose, leaving enriched cellulose. However, certain other white rot fungi such as, *Heterobasidium, Annosum, Irpex lactous* degrade the cell wall components simultaneously [24]. Lignin removal is important in the pulping and paper industry. This pulp treatment not only improves paper strength and remove low extractives but also reduce the energy consumption in the process of pulping. So, pretreatment of wood chips for mechanical and chemical pulping with white rot fungi has been developed. Bio bleaching is the bleaching of pulps using enzyme or lignolytic fungi that reduce the amount of chemical bleach required to obtain the desirable brightness of the pulps. Laccase mediated system has shown to possess the potential to substitute for chlorine containing reagents. Call and Call (2005) applied laccases as bio bleaching agents as they degrade lignin and decolourize the pulp. Bio bleaching of eucalyptus kraft pulp with certain while rot fungi in the presence of H_2O_2 resulted in significant reduction of kappa number with no change in viscosity suggesting a potential application of white rots [25]. Eugenio *et al.,* (2011) carried out the experiments on *Eucalyptus globosus k*raft pulps with laccase enzyme in the presence of aceto syringone as a netural mediator showed reduction in kappa number and increase of brightness without decreasing viscosity values, which was significantly as observed in the present investigation [26]. Selvam and Arungandhi (2013) in their studies recorded that bio bleaching and delignification of Hird Won Kraft Pulp three white rot fungi, *Trametes sp*¸ *Ganoderma sp*, and *Poria sp, r*educed the kappa number and increased the brightness of the pulp after 10 days of incubation [27].

Various studies on pretreatment of pulp by enzymes showed that they can decrease the amount of chemical required to attain same brightness in subsequent bleaching stages [28]. The utilization of lignolytic and xylanase enzymes resulted in easier bleaching in subsequent stages and better pulp brightness. The enzymatic hydrolysis of reprelipitated xylem on the surface of the fiber makes the fiber more permeable to lignin removal.

The potential applications of lignin degrading fungi and their enzymes in biotechnology has stimulated their investigation and the understanding of physiological mechanisms regulating enzyme synthesis in lignocellosic bioconversion could be useful for improving the technological process of edible and medicinal mushroom production [29,30]. Laccases are able to depolymerize lignin and delignify wood pulps, kraft pulp fibers and chlorine free in the bio pulpation process [31,32]. Strebotnik and Hammel (2000) studied the applications of white rots in the industry related to laccase mediator bleaching of kraft pulp and the efficiency of which has been proven in mill – scale trials [33]. This ability could be used in the future to attach chemically versatile compounds in the fiber surfaces and let recycled pulp for new use [34]. Lignin peroxidase and laccase are effective biocatalysts of choice for bleaching [35,36]. Lignin peroxidase and manganese peroxidase were reported

to be effective in decolourizing kraft pulp mill effluents [37]. Maijala *et al.* (2007) reported that the consumption of refining energy in mechanical pulping was reduced with manganese peroxidase pre-treatment with slight improvement in pulp properties [38,39]. They concluded that lignolytic enzymes are promising to replace the convential chemical processes of several wood based industries.

The introduction of some white rot strains (IZU - 154) to kraft bleaching made it possible to obtain bleached kraft pulp without the use of chlorine [40]. These bleached pulps had good optical and strength properties but unfortunately the use of fungal bleaching process is very slow and takes days instead of hours [41]. The direct use of an actively growing fungus for pulp bleaching is, therefore not feasible for industrial processes due to the time constraints and the degradation of the cellulose caused by cellulases secreted by the fungus [42]. The lignolytic enzymes rather than the fungus itself after a faster and more direct attack on the lignin structure, the laccase was only successful in reducing the lignin content of pulps in the presence of the living fungus, which indicated that the enzyme alone is not responsible for delignification [43]. Bourbonnais *et al.* (1995) reported that kraft pulp is delignified by laccase only in the presence of a mediator such as 2,2-azinobis (3-ethyl- benzthiazoline-6-sulphonate) (ABTS), but never by the laccase enzyme alone [44]. Thus, the ABTS has the ability to act as a mediator for laccase, thereby enabling the oxidation of non-phenolic lignin compounds that are not laccase substrates [45]. This mediator was found to prevent and ever reverse polymerization of kraft lignin and promotes the delignification of kraft pulp of laccase.

Manoharachary *et al.* (2005) reported that mushrooms alone are represented by about 41,000 species, of which approximately 850 species are recorded from India, and more than 2000 species of edible species are reported in the literature from different parts of the World [46]. Singer (1989) had reported 1320 species belonging to 129 genera under agaricales [47]. Extensive surveys an macro fungi were under taken and concluded that macro fungi are the associates of mycorhizal and determined the ecosystem dynamic of forests [48-50]. Numbers of reports are available on the diversity of white not fungi in various habitats, their lignolytic potentials and their role in the bio bleaching and bio pulping of UKP is wood based industries [51-55]. Ferraz *et al.,* (2008) visualized variety of novel technological advances and mechanistic basis for fungal bio bleaching and bio pulping. They selected very potential white rots and used strategies far the large scale production of lignolytic enzymes are used in the pretreatment of Kraft Pulp [56]. Singh and Chen (2008) screened different strains of *Phenerochaete* and identified few potential strains for the industrial purpose and also qualitatively and quantitatively different the efficiencies of those strains in the production of lignolytic enzymes [57]. Tran *et al.* (2013) identified few white rot fungi and used them as decomposers, and also in the bioremediation of industrial wastes of which they underlined the great contribution of *Trametes versicolor* in solving the problem related to degradation of toxic pollutants [58]. Andrew *et al.* (2013) surveyed the diversity and distribution of macro fungi in the Mount Cameroon and gathered the baseline information for the assessment of charges in biological diversity and used it as first step towards producing a check list of macro fungi [59]. Bindu *et al.* (2014) Krishna *et al.* (2015) collected variety of white and brown rot fungi from the forests of Andhra Pradesh and identified *Fomitopsis feei* as a potential brown rot fungus for the decolorization of effluents and their biomolecules were used as basic compounds for the manufacture of antibiotics [60,61]. Ram Prasad *et al.,* (2014) also surveyed the various habitats of Warangal town and collected good number of macro fungal strains, of which two strains of *Trametes* were proved to useful in the bleaching and pulping process [62]. The Exopolysaccharides produced from these fungi are identified as active bio compounds for the treatment of cancer. Kamali and Khodaparast (2015) reviewed the recent developments on pulp and paper mill wastewater treatment and identified the microbial enzymes as promoters of safe remediation strategy [63]. Li *et al.* (2015) and Hatice and Kasra (2016) used *Funalia trogii* and *Phanerochaete chrysosporium* respectively in the solid state fermentation to decolorize and remove heavy metals from the contaminated sites [64,65]. The role of fungal enzymes with special reference to laccases were studied for the cleavage of lignin in wood based industries for the improvement of brightness to the pulp and identified their activities in the environmental management [66,67]. Zhu *et al.* (2016) optimized the conditions of laccase production in the white rot fungus, *Pleurotus ostreatus* induced through yeast extract and copper for its use in biobleaching and biopulping of paper and pulp industries [68].

Conclusion

The conclusion of the present work indicated that the bio bleaching process is technologically feasible. The important aspect of economic analysis indicated that the biological treatment processes are

economically beneficial. Greater benefits can be realized by bio bleaching i.e. a reduction in chemicals, an increased throughput, environmental safety, health of work force etc. A large amount of effort has gone in to this research during the past 10 years to bring this technology to commercialization. However, many questions remain unanswered. The most important basic question is the molecular mechanism of bio bleaching. An understanding of the mechanism will facilitate the optimization of the process for mechanical, chemical and biological bleaching. The use of biological procedures for the kraft process is still an open research issue. Finally, the use of this technology for various other substrates with other diversified potential white rot fungal stains and their secretes of molecular mechanisms are to be investigated in future.

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