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Enzymatic Deinking of Waste Papers by Daldenia concentrica, Lepiota sp. And Trametes serialis

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Abstract: ***The accumulations of office waste papers were chemically recycled after deinking processes. The utilization of synthetic chemicals increases toxicity level leads our environment towards accretion of hazardous chemical substances. Three wood rot fungi Daldeniaconcentrica, Lepiota sp. and Trematesserialis were collected from Western Ghats of Tamil Nadu, India. The fungal isolates and their enzymes like LiP, MnP, Laccase and mixed enzymes were taken for deinking of waste papers (ink papers, photo copy paper and news paper). All the three fungi were recommended for the deinking of waste papers which can be recycled.***

Keywords: Enzymatic Deinking, Waste paper, Daldeniaconcentrica, Lepiota sp. , Trametesserialis

I. INTRODUCTION

Wood is formed of three most important components cellulose, hemicelluloses and lignin. Lignin constitutes the second richest group of biopolymers in the biosphere [1]. Lignin is extremely resistance towards chemical and biological degradation and provides mechanical resistance to wood.

Wood rotting fungi are an imperative component of forest bionetwork belongs to the order basidomycetes that play a part in the biodegradation of lignin in environment, which is indispensable for global carbon recycling [2]. White rot fungi can degrade lignin and can degrade environmental pollutants by many of their extracellular ligninolytic enzymes [3].Lignin degrading enzymes, lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase (Lac) play a key role in generating free radicals from lignin and oxidizable fungal metabolites such as oxalate, glyoxylate, malonate, hydroquinones and aryl alcohols. Some of the Lignin-modifying enzymes (LMEs) are lignin peroxidases (LiPs), manganese-dependent peroxidases (MnPs) and laccases. LiP and MnP oxidize nonphenolic aromatic compounds with high oxidation-reduction potentials from the major components of the lignin polymer. Lignin biodegradation by white rot fungi is an extracellular chemical event generating free radicals [4]. Enzymatic deinking can be applied on old newsprint and office waste, but it is more effective on the latter, because it contains large amounts of laser and xerographic inks that are more difficult to remove by conventional methods. The commercial cellulases used, are able to hydrolyse fines to release ink particles. The ink can be removed by chemical or air flotation. The secondary benefit of the enzyme treatment is that the stripping of fines from fibres results in improved drainage without reduction in strength properties [5]. The advantages of biobleaching includes reduced consumption of bio bleaching chemical, reduced organic halogen, improved pulp and paper pulp and paper quality improved brightness, reduced effluent toxicity and pollution load [6]. Pulp and paper production are the major industries in India, with a total capacity of over 3 million tons per annum. In paper making process, raw materials such as wood, bamboo, reeds, grasses, straw and bagasse are mechanically and chemically pulped by cooking in mixture of water, caustic soda, sodium sulphate and sodium carbonate. The pulp is then bleached using chlorine or similar oxidizing agents [7]. Enzymatic treatment by wood rot fungi can result better deinking without destructing the physical properties of paper products [8]. Though the wood rot fungi cause huge economic losses, novel investigations of these wood rot fungi can be used beneficially in a variety of new technologies for the welfare of the society. Several wood decay fungi play a pivotal role in biotechnological processes especially in the wood products, pharmaceutical and agricultural industries have selected and evaluated superior ligninolytic fungi for biological control, bio pulping and bio bleaching of wood chips, decolourization of dyes and deinking of waste water especially pulp and paper industry effluent treatment.

II. MATERIALS AND METHODS

A. Collection and identification of fungi

The fungi Daldeniaconcentrica, Lepiota sp. and Trametesserialis were collected from decayed wood of Western Ghats area of Tamilnadu and Karnataka, South India . The collection site was situated in the latitude of 11.58°S and longitude of 76.93°E at 420 ±

50M MSL. It receives rain fall of about 300 mm per year with high humidity and even temperature. The collected fungi were identified based on the suitable literature [9], [10].

B. Preparation Of The Culture Media

Fungal growth was taken and sterilized with 1% mercuric chloride solution, continuously washed with sterile distilled water and inoculated with 2% malt agar medium [11]. The fungal growth was sub cultured for 6 days at 37°C and they were maintained on malt agar slants.

C. Malt Agar Medium

Malt extract	-	20 g
Agar	-	20 g
Distilled water	-	1000 ml
pH	-	6.5

D. Inoculum

The spore suspension obtained from the malt agar plates were used as inoculums for further studies.

E. Preparation Of Spore Suspension

The fungi were grown in malt agar medium by dissolving 20 g of malt extract in distilled water and made up to 1000 ml. The pH was maintained as 6.5 at 37°C then the plates were flooded with sterile distilled water and brushed with camel hair brush smoothly without disturbing the mycelial growth and filtered through a sterile filter. The concentration of the filtrate was adjusted to 10^5 spores/ml and inoculum was used for further studies.

F. Fungal Culture And For Paper Treatment

The waste paper like ink, photo copy papers and news papers were obtained from various offices. Mycological broth (200 ml) in a conical flask (500 ml) added with a glass bead (2.5 cm dia) and waste papers (0.25%) was inoculated with fungal spore suspension (10^5 spores/ ml) and incubated with shaking (200 rpm) at 25°C for 5 days. After 5 days, the resulting suspension was inoculated (15% v/v) into 500 ml flasks containing sterile water (200 ml) and 1 or 2 per cent waste papers (dry weight basis). The flasks were incubated with shaking (200 rpm) at 25°C for 1 to 5 days [12].

G. Parameters Studied

The final pH, kappa number and brightness of the treated pulp were determined. The pH of the pulp solution was measured directly by using a pH meter, kappa number and brightness were estimated from standard hand sheets prepared from the pulp after harvest.

H. Preparation Of Hand Sheet

To prepare the hand sheets (2x4 cm size), the pulp suspension was filtered through a Buchner funnel vacuum. The residue was blotted and air dried for 24 h [13].

I. Production Of Ligninolytic Enzyme

The ligninolytic enzyme production, C- limited medium proposed by [14]. The enzymes lignin peroxidase (LiP), manganese dependent peroxidase (MnP) and laccase (Lac) were purified from the culture filtrate by acetone precipitation (66%v/v) and Sephadex G-100 Coloumn chromatography.

J. Treatment Of Waste Papers By Ligninolytic Enzymes

The waste papers (2.5 g dry weight) was incubated with various concentrations (5,10,15 U/ml) of mixed enzyme, LiP, MnP, (in sodium succinate buffer, 20mM; pH 4.5) and laccase (in phosphate buffer, 0.1M; pH 7.0) at 50ºC for 24 h After the incubation

period, the pulp was washed repeatedly with distilled water and hand sheets were made. The kappa number and brightness of the hand sheets were determined.

K. Lignin peroxidase (LiP)

The lignin peroxidase activity assay[15]. The assay solution contained.

Culture filtrate	-	1.0 ml
Sodium tartrate buffer (pH 3.5)	-	100 mM
Veratyl alcohol	-	0.4 mM
Fresh H_2O_2	-	0.3 mM

Immediately after adding H_2O_2, the change in optical density at 310 nm was recorded at 30 sec interval. The enzyme activity was expressed as U/ml (1U = µ mole of veratyl alcohol oxidised in 1 min).

L. Manganese dependent peroxidase (MnP)

Manganese dependent peroxidase assay [16]. The assay mixture consists of

Culture filtrate	-	1.0 ml
Phenol red	-	0.01 %
Lactate	-	25 mM
$MnSO_4$	-	100 M
Egg albumin	-	0.1 %
H_2O_2	-	100 µM in 1ml of 20 mM
Sodium succinate buffer, pH 4.5		

Reactions were carried out at 30°C for 5 min and terminated with addition of NaOH (40 µl). The optical density was measured at 610 nm. The enzyme activity was expressed as U/ml (1 U = change in OD/min at 610 nm).

M. Laccase

Laccase activity assay [17]. The reaction mixture consists of,

Culture filtrate	-	0.5 ml
Guaiacol	-	0.35 µl (0.035 % v/v)
Sodium acetate buffer 0.1M; (pH 5.0)	-	2.0 ml

The enzyme activity was expressed as change in optical density (OD) at 440 nm and expressed as U/ml (1U = change in OD/min at 440 nm).

N. Kappa Number

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 hand sheets 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 $KMnO_4$ solution (0.1 N), 100 ml of H_2SO_4 (4 N) was added and cooled to 25°C and immediately added to disintegrated hand sheet 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 procedure but without pulp. The kappa number was calculated by the formula

$K = p \times f / W$

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 pulp (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
Pf
K= ------ [0.0+0.013(25-t)]
W
Where,
t = actual reaction temperature in degree celsius.

O. Brightness

Brightness of the hand sheets were measured at 457 nm in a Perkin Elmer λ3B spectrophotometer equipped with a reflectance sphere.

III.RESULTS

The present study conducted to explore the most effective enzymes to serve as an innovative approach for enhancing the deinking of waste papers and to analyse the effectiveness on a specific type of activity driven by the ligninolytic enzymes.

A. Deinking of waste papers by ligninolytic enzymes

LiP, MnP and laccase a mixture of the enzymes of *Daldeniaconcentrica, Lepiota sp.* and *Trametesserialis* were used at various concentrations (5, 10 and 15 U/ml) fordeinking of waste papers. The results of the deinking of various waste papers by the three fungal enzymes were shown in Fig.1. Kappa number and brightness were considered as parameters for quality determination. Fungal treatment reduces the pH of the reaction mixture.

1) *Ink papers:* Ink papers were the waste papers that were obtained from offices and schools. For the treatment of ink paper the taken control shows mixed enzyme, LiP, MnP and laccase Kappa number 9.1 respectively and mixed enzyme and laccase shows Brightness 59.01 ISO units respectively, LiP 46.32 and MnP 46.31 (Table. 1)
2) *Daldeniaconcentrica:* In Ink paper the mixed enzyme preparation at 15 U/ml concentrations, *Daldeniaconcentrica* reduced the kappa number by 29.67 per cent and laccase reduced upto 50.77 per cent, but in LiP and MnP treatments maximum reduction 44.51 and 59.34 respectively were caused by 10 U/ml concentrations. In pulp brightness, mixed enzymes and LiP at 10U/ml concentration yielded maximum increase of 12.57 and 13.43 per cent respectively, Whereas maximum brightness were observed 12.89 and 11.86 per cent respectively for MnP and laccase treatment at 15 U/ml enzyme concentration .
3) *Lepiota*sp: PIn *Lepiota sp.* treatment of the enzyme preparations at 15U/ml concentration yielded maximum reduction in kappa number 29.67, 50.77, 50.77 and 44.51 per cent respectively for mixed enzyme, LiP, MnP and laccase; mixed enzyme yielded high brightness 13.73 per cent at 15 U/ml concentration, whereas LiP, MnP and laccase 12.44, 12.22 and 14.79 per cent respectively at 10 U/ml concentration and brightness at high concentration of 10 U/ml.
4) *Trametesserialis:* In Trametesserialis, treatment of the enzyme preparations at 15U/ml concentration, yielded maximum reduction in kappa number 44.51, 66.70 per cent respectively for mixed enzyme, MnP. For laccase 66.70 and LiP was 59.34 per cent at 10U/ml concentration; mixed enzyme yielded high brightness 14.32 ISO units at 15 U/ml concentration, whereas LiP, MnP 14.01, 13.09 per cent respectively at 10 U/ml concentration and brightness at high concentration of 15 U/ml; laccase yielding high brightness of 14.29 per cent respectively.
5) *Photocopy papers :*Photocopy papers are widely used now a day by all sectors mainly by educational institutions. For the treatment of ink paper the taken control shows Kappa number 8.43 for mixed enzyme, LiP, MnP and laccase respectively and brightness was 36.06 ISO units (Table. 2).
6) *Daldenia Concentrica: Daldeniaconcentrica* mixed enzyme reduced the kappa number by 40.09 per cent at 10 U/ml concentration; but brightness was increased to higher level 52.25 per cent by 15 U/ml enzyme. In LiP treatment, maximum reduction in kappa number 24.08 per cent and increase in brightness 40.32 per cent were observed at 10 U/ml concentration. In MnP and laccase treatment, maximum reduction in kappa number 32.03 and 48.04 % was observed at 10 U/ml concentration, brightness was increased to the maximum level 42.37 per cent by 10 U/ml concentration of laccase, whereas MnP was essential in high concentration 41.82 per cent was increased.

7) *Lepiota sp.:* In Lepiota sp. mixed enzyme was treated to pulp, maximum reduction in kappa number 56.11 per cent was observed at 15 U/ml concentration, but brightness was increased to the higher level 43.59 per cent at 10 U/ml concentration. In LiP treatment, the kappa number was reduced only by 24.08 per cent at both 10 and 15 U/ml concentration, whereas MnP at 10 U/ml concentration decreased the kappa number by 40.09 per cent and laccase reduced up to 56.11 per cent and at 15U/ml concentration. All the enzyme preparations increased the brightness of mixed enzyme and laccase at 10 U/ml concentration of brightness 43.59, 44.43 per cent and LiP 39.02 and MnP 44.56 at 15U/ml concentration.

B. Trametes Serialis

In Trametesserialis, higher concentration of enzymes yielded higher reduction in kappa number and increase in brightness. In 15 U/ml of mixed enzymes was reduced the kappa number by 56.11 per cent; LiP at this concentration reduction the kappa number by 32.03 per cent and MnP and laccase reduced by 48.04 and 64.06 per cent respectively. Mixed enzyme, LiP, MnP and laccase at 15 U/ml concentration increased the brightness by 51.36, 44.98, 53.58 and 53.05 per cent respectively.

1) *News Papers:* News papers were used all over the world. For the treatment of news paper the taken control shows Kappa number 10.45 for mixed enzyme, LiP, MnP and laccase respectively and brightness was 34.95 ISO units (Table. 3).
2) *Daldeniaconcentrica:* The enzymes of Daldeniaconcentricaat 15U/ml concentration yielded maximum reduction in kappa number; mixed enzyme yielded 51.67 per cent respectively Whereas LiP, MnP and laccase yielded 38.76, 45.17 and 71.00 per cent respectively. The brightness was increased to a maximum level of 17.91 and 19.34 per cent respectively by mixed enzyme and laccase at 15 U/ml concentration; whereas in LiP and MnP, the yields were at the maximum of 7.81 and 15.22 per cent at 5, 10 U/ml concentration respectively.
3) *Lepiota*sp.: In Lepiota sp. treatment also 15 U/ml concentration of enzymes yielded maximum reduction in kappa number 45.17, 32.25, 45.17 and 57.13 per cent respectively for mixed enzymes, LiP, MnP and laccase. Similarly, maximum increase in brightness was observed at this higher concentration, mixed enzymes 17.57 brightness at 15 U/ml concentration and 13.28 to 20.23 LiP and laccase at 15 U/ml concentration except in that of MnP, whereas maximum of only 10.76 increase was observed at 15 U/ml concentration.
4) *Trametes Serialis:* The Trametesserialisenzyme treatment also, higher concentration of enzymes yielded higher reduction in kappa number. Mixedenzyme and MnP at 15 U/ml concentration reduced the kappa number by 51.67 per cent, LiP reduced by 32.25 per cent and laccase by 64.59 per cent. In higher increased brightness values were obtained with 10 U/ml concentration of enzymes; mixed enzyme increased the brightness by 15.28 per cent, whereas LiP, MnP and laccase increased the brightness by 11.59, 6.95 and 15.57 per cent respectively.

IV. DISCUSSION

The application of the enzymes were plays a vital role in paper recycling. The enzyme has the capacity to reduce or that eliminates the usage of chemicals in deinking processes. The physical properties were improved while substituted with enzymes like increased brightness, decreases the residual ink count and freeness of the recycled pulp. The adsorption deinking was an innovative notion for ink amputation from suspensions by means of polymer particles instead of air bubbles in a process with high efficiency, concerning the consumption of energy and water and the influence of different papers and inks, of the deinking solutions and the duration of deinking. The process is characterized not only by the colour of polymer particles, but also by the properties in suspension such as charge, turbidity or particle size. Further it is shown, that dynamic surface tension is a useful tool to characterize not only the surfactant in suspension, but also its interaction with other substances. The effectiveness of adsorption deinking is strongly influenced by the properties in suspension which dependupon on the type of paper [18]. Fungal laccases are believed to play a variety of roles, such as, morphogenesis, pathogenesis, and lignin degradation. As an oxidase, laccase is used in many agricultural, industrial, and medicinal applications [19].

The important source of recycled paper is a raw material for the pulp and paper industry. The major problems are the ink needs to be removed from the paper and also reused for making white paper. Hence recycled paper was treated with different deinking processes based on detergents and separation of ink particles by floating and the particles are concentrated at the surface and removed. The ink initiates tensions in the cellulose, which make it more amorphous than the surrounding material. Cellulases attack the amorphous cellulose faster than the more crystalline material. The ink can be removed easily, since part of the cellulose close to it is removed or weakened the hypothesis for of enzymatic deinking of fungi. Recycling industries were using expensive instruments to offer deinking by various techniques [20,21].

Recycling of waste papers was considered as essential to conquer increasing pollution hitch[22].The deinking process can be substantially improved by the addition of various enzymes to the detergent mixture. The commercial use in many countries, actually enzymatic deinking is one of the most common enzymatic techniques in the pulp and paper industry. The detergents and the enzymes must be carefully chosen in order to avoid enzyme inactivation. The exact composition of the industrial enzyme preparations used for deinking was industrial secrets, but hard fungal amylases (starch degrading enzymes) celluloses and to some extent, lipases and hemicelluloses are generally included. Lipases probably attack fatty structures in the ink itself, whereas the other enzymes are believed to attack either the starch used in paper coating or the cellulose around the printed area. Recycling of recovered paper has become an important issue in the pulp and paper industry of diminishing wood supply and environmental concerns over deforestation [23]. It contributes to preserve scare forests resources and promote overall water and energy conservation. Internationally one third of the paper is made from recycled fibers [24]. The removal of the printing ink from the used paper is one of the most important processes in recycling of paper [25]. The deinking of newspapers, photocopy papers and ink papers by three white rot fungi Fomeslividus, Thelephora sp. and Trametesversicolor were reported [26].Phanerochaetechrysosporium was able to degrade various pollutants and possess peroxidases enzymes and having lignin degrading capacity [27].

V. CONCLUSION

The enzymatic deinking of waste papers by three effective lignolytic fungi was carried out in the present study. The deinking of waste papers by ligninolytic fungi shows effective deinking. Trametesserialis reduced deinking activity on the ink paper was upto 66.70 per cent in 15 U/ml concentration of MnP , laccase and then brightness was increased to 59.34 ISO units at 10 U/ml concentration of Daldeniaconcentricain MnP; In Photocopy paper, the Trametesserialis were reduced kappa number was 64.06 per cent at 15 U/ml concentration in laccase and brightness was increased to 53.58 ISO units of MnP was at 15 U/ml concentration. In news paper, Daldeniaconcentricathe kappa number was reduced to 71.00 per cent at 15 U/ml concentration in laccase and in Lepiota sp. brightness was increased to 20.23 ISO units at 15 U/m concentration in laccase were more effective. Therefore all these fungi were found as potential application in the waste paper recycling.

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Table - 1 : Deinking of ink papers by ligninolytic enzymes

Enzyme conc (U/ml)	Mixed enzyme		LiP		MnP		Laccase	
	Kappa number	Brightness ISO units	Kappa number	Brightness ISO units	Kappa number	Brightness ISO units	Kappa number	Brightness ISO units
Control	9.1±0.9	59.01±0.6	9.1±0.7	46.32±1.0	9.1±0.9	46.31±0.9	9.1±0.8	59.01±1.0
D.concentrica								
5	7.75±0.9	63.63±0.5	5.73±0.6	50.99±1.2	4.38±1.0	51.23±0.7	7.08±0.9	65.80±0.5
	(-14.83)	(-7.82)	(-37.03)	(-10.08)	(51.87)	(-10.62)	(22.20)	(-11.51)
10	7.08±0.9	66.43±0.4	5.05±0.8	52.54±0.4	3.70±0.9	52.06±0.8	5.05±0.9	65.61±1.2
	(22.20)	(-12.57)	(44.51)	(-13.43)	(59.34)	(-12.42)	(44.51)	(-11.18)
15	6.4±1.2	64.51±0.9	5.05±1.3	52.50±0.5	3.70±0.6	52.28±1.2	4.48±0.9	66.01±0.8
	(29.67)	(-9.32)	(44.51)	(-13.34)	(59.34)	(-12.89)	(50.77)	(-11.86)
Lepiotasp								
5	7.75±0.5	65.78±0.1	5.73±0.5	50.24±0.6	5.05±1.2	51.20±0.8	7.08±1.2	66.22±0.7
	(14.84)	(-11.47)	(37.03)	(-8.46)	(44.51)	(-10.56)	(22.20)	(-12.22)
10	7.08±0.8	66.37±0.4	5.05±0.9	52.08±0.9	5.05±1.1	51.97±0.7	5.73±0.9	67.74±0.9
	(22.20)	(-12.47)	(44.51)	(-12.44)	(44.51)	(-12.22)	(37.03)	(-14.79)
15	6.4±0.8	67.11±0.9	4.48±1.3	52.08±0.7	4.48±0.8	51.92±0.9	5.05±1.1	66.90±0.9
	(29.67)	(-13.73)	(50.77)	(-12.44)	(50.77)	(-12.11)	(44.51)	(-13.37)
T.serialis								
5	6.4±0.4	64.85±1.4	4.38±1.0	52.81±1.3	5.05±0.6	52.15±1.0	7.08±0.8	64.80±1.0
	(29.67)	(-9.90)	(51.87)	(-14.01)	(44.51)	(-12.61)	(22.20)	(-9.81)
10	5.73±0.8	65.70±0.7	3.70±0.5	51.40±0.6	4.48±1.0	52.37±0.6	4.48±1.0	66.06±1.4
	(37.03)	(-11.34)	(59.34)	(-10.97)	(50.77)	(-13.09)	(50.77)	(-11.95)
15	5.05±1.0	67.46±0.9	3.70±0.9	50.48±1.3	3.03±1.0	51.33±0.7	3.03±0.9	67.44±0.3
	(44.51)	(-14.32)	(59.34)	(-8.98)	(66.70)	(-10.84)	(66.70)	(-14.29)

LiP : Lignin peroxidase; MnP : Manganese dependent peroxidase; Dc : *Daldeniaconcentrica* ; L : *Lepiota* sp. ; TS : *Trametesserialis*
Values are mean of three replicates ± Standard deviation;
Values in parentheses are percent increase (+) or (-) over control

Table – 2: Deinking of photo copy paper by ligninolytic enzymes

Enzyme conc (U/ml)	Mixed enzyme		LiP		MnP		Laccase	
	Kappa number	Brightness ISO units	Kappa number	Brightness ISO units	Kappa number	Brightness ISO units	Kappa number	Brightness ISO units
Control	8.43±1.0	36.06±1.0	8.43±1.2	36.06±3.0	8.43±1.0	36.06±2.0	8.43±2.0	36.06±1.1
D.concentrica								
5	5.73±1.2	51.45±1.2	7.08±1.1	47.31±1.0	6.40±1.2	50.50±1.0	5.05±1.0	54.61±1.2
	(32.03)	(-42.68)	(16.01)	(-31.06)	(24.08)	(-40.04)	(40.09)	(-51.44)
10	5.05±1.1	54.23±1.2	6.40±1.3	50.60±1.1	5.73±1.0	51.14±1.1	4.38±0.9	51.34±1.0
	(40.09)	(-50.09)	(24.08)	(-40.32)	(32.03)	(-41.82)	(48.04)	(-42.37)
15	5.53±0.7	54.90±1.5	7.08±1.3	49.35±1.0	5.73±1.2	52.58±1.1	4.38±0.7	50.75±1.1
	(34.40)	(-52.25)	(16.01)	(-36.86)	(32.03)	(-38.99)	(40.09)	(-40.74)
Lepiotasp								
5	5.05±1.2	50.36±1.1	7.08±1.4	48.15±1.7	5.73±1.6	50.12±1.2	5.05±1.0	47.07±1.4
	(40.09)	(-39.66)	(16.01)	(-32.03)	(32.03)	(-38.99)	(40.09)	(-30.53)
10	4.38±1.4	51.78±1.0	6.40±1.6	49.20±1.5	5.05±1.4	51.58±1.0	4.48±1.1	52.08±1.4
	(48.04)	(-43.59)	(24.08)	(-36.44)	(40.09)	(-43.04)	(46.86)	(-44.43)
15	3.70±1.1	51.12±1.0	6.40±1.2	50.13±1.1	5.05±1.2	52.13±1.2	3.70±1.3	49.75±1.1
	(56.11)	(-41.76)	(24.08)	(-39.02)	(40.09)	(-44.56)	(56.11)	(-37.96)
T.serialis								
5	5.05±1.0	49.61±1.7	7.08±1.4	50.91±1.9	5.05±1.1	53.13±1.3	4.38±1.4	52.59±1.4
	(40.09)	(-37.58)	(16.01)	(-41.18)	(40.09)	(-47.34)	(48.04)	(-45.84)
10	4.48±1.2	53.87±1.3	6.40±1.4	51.31±1.3	5.05±1.3	54.61±1.1	3.70±1.3	52.57±1.7
	(46.86)	(-49.39)	(24.08)	(-42.29)	(40.09)	(-51.44)	(56.11)	(-45.78)
15	3.70±0.6	54.58±1.8	5.73±1.5	52.28±1.2	4.38±1.1	55.38±1.2	3.03±1.1	55.19±1.5
	(56.11)	(-51.36)	(32.03)	(-44.98)	(48.04)	(-53.58)	(64.06)	(-53.05)

LiP : Lignin peroxidase; MnP : Manganese dependent peroxidase; Dc : *Daldeniaconcentrica* ; L : *Lepiota* sp. ; TS : *Trametesserialis*
Values are mean of three replicates ± Standard deviation;
Values in parentheses are percent increase (+) or (-) over control

Table - 3 :Deinking of Newspaper by ligninolytic enzymes

Enzyme conc (U/ml)	Mixed enzyme		LiP		MnP		Laccase	
	Kappa number	Brightness ISO units	Kappa number	Brightness ISO units	Kappa number	Brightness ISO units	Kappa number	Brightness ISO units
Control	10.45±1.0	34.95±0.3	10.45±2.0	34.95±3.0	10.45±2.0	34.95±1.0	10.45±1.0	34.95±2.0
D.concentrica								
5	7.75±1.7	39.03±1.9	9.78±1.1	37.68±1.7	8.43±1.1	38.70±0.9	6.40±1.0	39.33±1.7
	(25.84)	(-11.67)	(6.41)	(-7.81)	(19.33)	(-10.73)	(38.76)	(-12.53)
10	7.08±1.1	40.19±1.1	8.43±1.1	37.52±1.4	6.40±1.1	40.27±1.0	4.48±1.0	40.52±1.5
	(32.25)	(-14.99)	(19.33)	(-7.35)	(38.76)	-(15.22)	(57.13)	(-15.94)
15	5.05±1.4	41.21±1.5	6.40±1.4	37.42±1.2	5.73±1.1	39.50±0.8	3.03±1.0	41.71±1.2
	(51.67)	(-17.91)	(38.76)	(-7.07)	(45.17)	(-13.02)	(71.00)	(-19.34)
Lepiota sp.								
5	8.43±1.1	37.04±1.0	8.43±1.2	36.09±1.5	8.43±1.2	35.95±0.7	7.08±1.0	37.74±1.2
	(19.33)	(-5.98)	(19.33)	(-3.26)	(19.33)	(-2.86)	(32.25)	(-7.98)
10	7.08±1.1	40.10±1.3	7.75±1.1	38.35±1.1	7.08±1.3	37.19±0.8	5.73±1.0	40.61±1.7
	(32.25)	(-14.74)	(25.84)	(-9.73)	(32.25)	(-6.41)	(45.17)	(-16.19)
15	5.73±1.2	41.09±1.2	7.08±1.2	39.59±1.1	5.73±1.1	38.71±1.0	4.48±0.9	42.02±1.4
	(45.17)	(-17.57)	(32.25)	(-13.28)	(45.17)	(-10.76)	(57.13)	(-20.23)
T. Serialis								
5	7.75±1.3	40.53±1.4	9.10±0.9	35.49±0.8	7.75±1.3	36.42±1.0	6.40±1.0	41.29±1.5
	(25.84)	(-15.97)	(12.92)	(-1.55)	(28.84)	(-4.21)	(38.76)	(-18.14)
10	6.40±1.1	40.29±0.9	8.43±1.3	39.00±1.2	6.40±1.2	37.38±0.7	5.05±1.0	41.44±1.2
	(38.76)	(-15.28)	(19.33)	(-11.59)	(38.76)	(-6.95)	(51.67)	(-15.57)
15	5.05±1.3	39.03±1.3	7.08±1.2	38.40±1.2	5.05±1.1	37.19±0.8	3.70±1.0	39.00±1.2
	(51.67)	(-11.67)	(32.25)	(-9.87)	(51.67)	(-6.41)	(64.59)	(-11.59)

LiP : Lignin peroxidase; MnP : Manganese dependent peroxidase; Dc : *Daldeniaconcentrica* ; L : *Lepiota* sp. ; TS : *Trametesserialis*
Values are mean of three replicates ± Standard deviation;
Values in parentheses are percent increase (+) or (-) over control

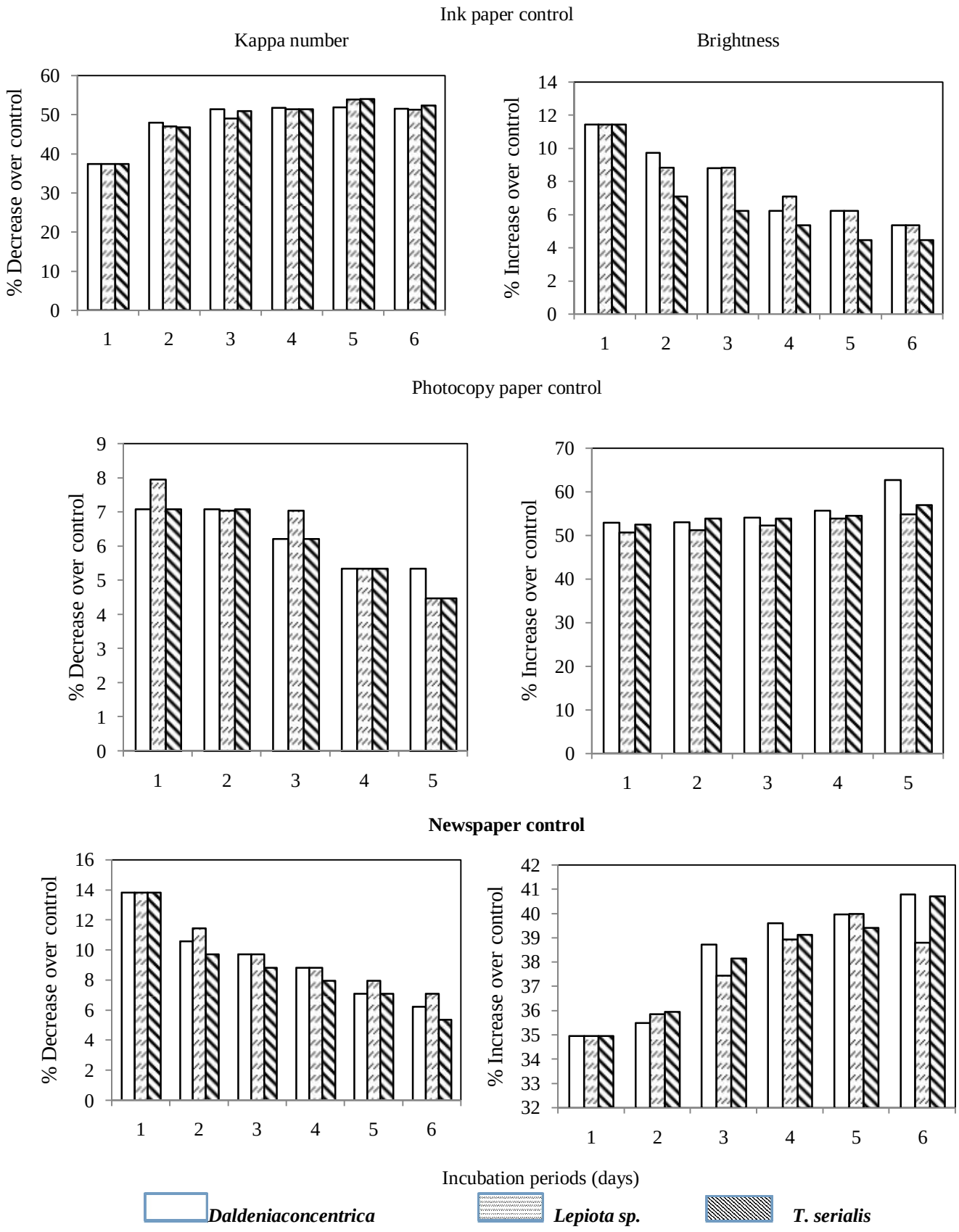
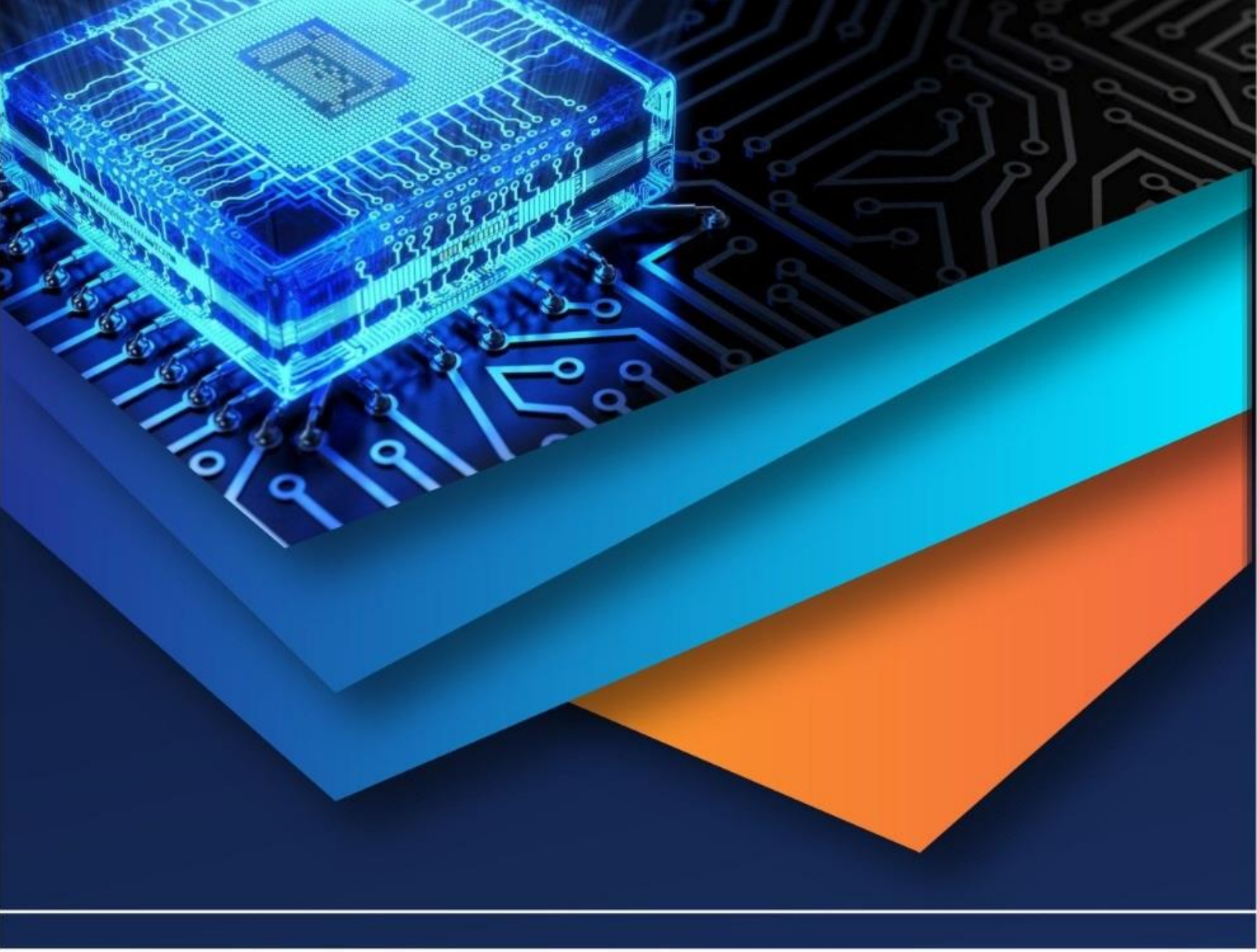


Figure - 1 : Effect of fungal treatment on Kappa number % decrease over control and brightness % increase over control of Waste papers



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