Treatment of cardboard industrial effluent in nutrient amended and non-amended condition for physico-chemical parameters and mutagenicity reduction

Shweta Kulshreshtha[1], Nupur Mathur[2], Pradeep Bhatnagar[3]

[1]Amity Institute of Biotechnology, Amity University Rajasthan, Jaipur. Email: shweta_kulshreshtha@rediffmail.com

[2]Department of Zoology, University of Rajasthan, Jaipur. Email:nupurmathur123@rediffmail.com

[3]The IIS University, Mansarovar, Jaipur. Email: pradeepbhatnagar1947@yahoo.com

Corresponding author:

Dr.Shweta Kulshreshtha

A-200, Vaishali Nagar, Jaipur-302021, Rajasthan, India.

Mobile: +919460553136

E-mail: shweta_kulshreshtha@rediffmail.com

Abstract

Pupose: Today, cardboards have become a necessary part of contemporary life due to their durability and strength. Cardboard production is highly water intensive process that requires water in each step, thus increasing the water requirement and generating relatively high amount of effluent. These effluents pose a *serious threat to the environment* and therefore, *need to be treated and managed* in a proper way. This study was conducted to determine if the fungal treatment process requires the supplementation of effluent for effective treatment. In most of the cases, effectiveness of treatment is decided by reduction in only physico-chemical parameters. However, *mutagenicity is also an important criterion* to find out the effectiveness of the treatment but that is meagerly reported so far.

Methods: Keeping this in mind, effluent of cardboard industries was treated with fungi and pre- and post-treatment physico-chemical parameters and mutagenicity was analyzed using standard protocols of APHA (1995) and Ames test. In Ames test,*Salmonella typhimurium* TA 98 and TA 100 were used to detect the presence of frameshift and basepair mutagens respectively.

Results: The results of this study showed that pH was shifted from acidic to basic scale. Colour was not reduced significantly while mutagenicity reduced significantly by *P.florida* in the presence and absence of nutrients. For reducing COD, *Phanerocheatechrysosporium* and *Pleurotus citrinopileatus* in nutrient amended condition was found to be effective.

Conclusion: Therefore, none of them were able to treat the effluent efficiently for reducing physicochemical parameters and mutagenicity in all conditions. In future, a mixed consortium of fungi can be used to reduce various parameters and mutagenicity and for complete treatment of cardboard industrial effluent.

Keywords: aerobic treatment, mutagenicity, Ames test, cardboard industries, effluent treatment, fungal treatment, mutagenicity reduction.

1. Introduction

Cardboard is a thick layer of paper, made up of waste papers and discarded cardboards. This is not only stiff,*rigid, strong and light weight material, but also versatile, recyclable and dynamic form*, used for packaging purpose. Cardboards can be cut and folded into a variety of shapes and sizes to make packaging boxes of different capacities and shapes. This is very effective material for packaging due to its efficiency in protecting the product against damage by shock. Millions of tons of cardboards are used every year to protect, display and transport of products. Its application provides all sorts of benefits and solutions in our routine life. There are many types of cardboards with different properties and strengthsthat offer many combinations for designing of packaging material with different characteristics, strength and performances. This utilization is associated with an increase in demand and production, which further leads to the establishment of cardboard industries.

Waste paper and discarded cardboards are used as the most common raw material for making cardboard by recycling process.Hence, these *industries not only make the cardboard, but also manage the waste accumulation* in the area. These are thought to be environmental friendly industries due to adoption of recycling technology. Pulp can be produced by waste papers and cardboards using pressure and mechanical forces. At every step of recycling, pulp fibres become too short to be recycled. All steps of cardboard makingprocess require a huge amount of water which is later discarded as effluent in the drainages. After processing of cardboards, shortened pulp residuesare discharged with the effluent and causes pollution[1].This coloured effluent cause serious harm to aquatic biota due to the reduction of sunlight penetration in aquatic bodies.

Cardboard wastes and effluents were used for the development of briquettes [2] and polyhydroxyalkanoates[3] production respectively. Amatand colleages [4] reported the detailed study of the degradation products after ozone and UV treatment. However, there *is scarcity of reports on the toxicity or mutagenicity of the effluents.* Besides, the effect of treatment methods on the various parameters as well as mutagenicity is meagerly reported [5, 6] According to Punziand colleagues [5] monitoring of mutagenicity or toxicity is crucial and should be used as an important parameter to decide the success of a treatment strategy which further helps in the management of this waste. Therefore, the treatment of cardboard industrial effluents and their physicochemical characterization and mutagenicity profile is an important and an unplumbed territory.

Some *interesting and unique points must be kept in mind before using this effluent for treatment*: (1) Cardboard industries (CI) use waste paper or cardboards instead of wood to obtain the pulp, and therefore, the composition of the effluents is not exactly the same as the paper mills have [4]. (2) Many cardboard industries do not use chemicals or bleaching agents for producing cardboards, yet brown colour is present in the effluent possibly due to the release of chemicals, inks, and toxic substances in the water during the pulp making process. (3) Chemicals are not used by the industries and therefore, toxicity of the effluents has not been reported so far. Therefore, this seems an interesting problem that needs to be investigated.

2. Material and methods

2.1 Sampling site

The present study focuses on the cardboard industries which are located in the *Sanganer area*, near Jaipur city, India. These industries recycle a variety of paper and discarded cardboards into cardboard sheet with the help of pulping machine and sheet making machines. A very huge amount of water is used in sheet formation on cardboard machines. This water contains very high COD and brown colour due to the release of dyes, chemicals, and various inorganic and organic compounds, during the pulping process [7].This water is discharged as effluent in the Amani Shah drainage.

These *compounds may be toxic* and when release with the effluent, makes the water body toxic. The seasonal changes in physicochemical parameters assessed earlier [7] revealed that these were found to be much above the discharging limit of [8] in every season. There is *no treatment strategy employed so far* for treating cardboard industrial effluent. Therefore, this concern about the treatment and management of this waste has created our interest in this field.

This study aimed at assessing the effect of fungal treatment not only on physicochemical parameters but also on mutagenicity of cardboard industrial effluent. Both were observed in nutrient amended and non-amended conditions before and after the treatment. In this study, effluent was neither diluted nor sterilized. These were treated directly in raw form as obtained from industries.

2.2 Collection and physico-chemical characterization of effluent:

The effluent samples for this study were *collected fromdifferent cardboard industries located in Sanganer*, Jaipur, India for the period of one year. The brown colored effluent was generated mainly from the pulping process and machine based cardboard sheet making process. Effluent samples of cardboard industries were collected in pre-sterilized plastic containers and immediately transferred to laboratory to *analyze physico-chemical parameters* as given in APHA [9]. All samples were stored at 4ºC to prevent its degradation by indigenous microbes until further use. However, before use for treatment, samples thawed to room temperature. This effluent was also assessed for its pre-treatment and post-treatment physicochemical parameters, such as pH, COD and colour.

2.3 Mutagenicity assay/ Ames assay

This is *Salmonella*microsome reversion (microbe based) assay was used to assess the pre- and post- treatment mutagenicity of the samples by using two strains *Salmonella typhimurium*: (i) TA 98 – for detecting frameshift mutagens (ii) TA 100- for detecting basepair mutagens.

All effluent samples were tested in their crude natural state without dilution. This assay was conducted using the *plate incorporation procedure* described by [10].This is prokaryotic cell based assay and therefore, need to incorporate the system to assess the level of mutagenicity in eukaryotic system in order to incorporate an important aspect of mammalian metabolism into the *in-vitro* test. This was done by adding hepatic S9 fraction of mouse in the system. Samples were tested on duplicate plates at five dose levels (2 µl, 5 µl, 10 µl, 50 µl and 100 µl) of individual samples. In this assay, 2-nitrofluorine (1 µg/plate: 104 revertants) and sodium azide (1 µg/ plate: 594 revertants) were used as positive and negative controls. Metabolic activation system S9 mix was prepared from un-induced liver of mouse as described by Privaland Mitchell [11] and added in the plates. This showed the increase in the number of revertants as TA 98 (1 µg/plate: 481) and TA 100 (1 µg/plate: 897). Di-methyl sulfoxide (DMSO) was added to observe spontaneous revertants in the negative control plates. All readings were taken in duplicates.

2.4 Fungal culture and maintenance:

The main constituent of *cardboard industrial effluent is cellulolytic pulp fibres which are rich in pulp fibres*, and therefore, only cellulolytic strains were selected for the present study.Strains employed in the present study, i.e. *Phanerochaetechrysosporium*, *Pleurotusflorida*and *Pleurotuscitrinopileatus*,were procured from various sources mentioned in Table 1.These strains were sub-cultured on Potato Dextrose Agar medium (PDA) and then preserved at 4°C until use.

Table 1: Fungal cultures used in the study, their source and maintenance

S. No.	Fungal Culture	Obtained from	Growth condition	Incubation temperature	Incubation period	subculture	Special features
1.	*Phanerochaetec*	MTCC,	Aerobic	35±2°C	7 days	30 days	Degrade

	hrysosporium(MTCC strain no.787)	Chandigarh					pulp and paper mill waste water
2.	*Pleurotus citrinopileatus*	Agricultural research station, Durgapura, Jaipur	Aerobic	35±2°C	7 days	30 days	Cellulolytic, edible fungi
3.	*Pleurotus florida*	NRCM, Solan	Aerobic	35±2°C	7 days	30 days	Cellulolytic, edible fungi
4.	Activated sludge	Brahmpuri Sewage treatment plant	Aerobic	35±2°C	48 h	Recollection after 30 days	Routinely used for treatment of waste water in Brhampuri sewage treatment plant, Jaipur.

2.5Cellulase activity test:

The fungi were *cultivated on CarboxyMethyl Cellulose (CMC) medium*which was prepared as given by [12]. Plates were incubated for different time periods i.e. 48 h, 72 h and 96 h at 37± 1ºC. Thereafter, plates were stained with 1% Congo red dye (15 min.), followed by destaining with 1M NaCl solution for 20 min. Colony diameter and zone clearing diameter were measured for observing the cellulose activity. *Cellulase activity of fungi on CMC agar*, was recorded as clear zone ratios = clear zone diameter/ colony diameter [13]. This is called index of Relative Enzyme Activity (I_{CMC}). This activity was also recorded for the known cultures of fungi used for the treatment of effluents.

2.6Effluent treatment with white rot fungi (WRF)

Cardboard industrial effluent was treated with 3 different white rot fungi in aerobic condition. In this study, effluent was neither diluted nor sterilized. These were treated directly in raw form as obtained from industries. 400ml amount of each effluent was taken separately in eight sterilized 1l Erlenmeyer flasks. All 8 flasks were divided in the two sets of 4 flasks. Out of two groups of effluent containing flasks, glucose (1g/l) as carbon and ammonium chloride (0.25g/l) as nitrogen source was added to one group of 4 flasks (nutrient amended effluent)[14-16]. Another group of flasks was used without adding any nutrient (non-amended effluent).

All fungi were grown on Potato Dextrose Agar (PDA) for 7 days for inoculation of flasks. Fungi were inoculated in the form of four mycelial discs of 0.5cm diameter in each flask, which were cut out with the help of a borer (0.5cm diameter) from the zone of active growth on a Petridish[17]. In all the assays, flasks were incubated in shaken (120 rev/min) condition for 12 days at 37 ± 1°C [18]. Withdrawals of samples were made on every 3rd day of experiment till 12th day. Readings were reported as % decrease or increase in parameters.

Cardboard industrial effluent was also inoculated with activated sludge which was collected from a sewage treatment plant, located at Brahmpuri, Jaipur, Rajasthan. This was done to find out the effect of the most employed technology on physico-chemical parameters and mutagenicity.

Control

Two controls of effluent samples were used in the present study. One was amended with nutrient while the other was not. In both of these effluent containing flasks, fungus was not added. These controls were used to compare the effect of fungi in nutrient amended and non-amended effluent with their respective controls.

2.7Post-treatment analysis

After fungal treatment, physico-chemical and mutagenicity was analyzed using the same protocol.

2.7 Statistical Analysis

In the present study, significant treatments and withdrawls were compared from control by using two way ANOVA. Null hypothesis was rejected ifFcal>Ftab. And critical difference in treatment and time period was calculated. After this post-hoc tests was performed using difference matrix for treatment and sample withdrawal days to find out the most significant treatment and number of days required for effective treatment.

In the case of mutagenicity analysis of data was done in two ways: (1) *Statistical approach*: in this approach, two way ANOVA was used only at highest dose level i.e. 100 µl to calculate the effect of treatment at specific dose level. (2) *Non-statistical approach*: This is based on the calculation of mutagenicity ratio. Mutagenicity ratio is the ratio of average induced revertants on test plates (spontaneous revertants plus induced revertants) to average spontaneous revertants on negative control plates (spontaneous revertants). Genotoxicity ratio of 2.0 (two fold) or more is regarded as a significant indication of genotoxicity and the compound is considered significantly mutagenic [19].

3. Result and discussion:

Cardboard industries recycle waste paper and cardboards and therefore, are the demand of the era for sustainable development. The only drawback of these recycling based cardboard industries is the requirement and release of high amount of water in the environment which cause pollution. In the present study, the detailed analyses of the physicochemical parameters were done for the period of one year and results are depicted in Table 2. Temperature of the *effluent varies and depends on the season while the variation in COD, BOD and colour depending upon the raw material used for making cardboard*. The pH of the effluent was found to be slightly acidic, which was contrary to the previously published report byAmatand colleagus [4]. All parameters were found above the discharging limits of CPCB [8] revealing unsuitability of the effluent for discharging into the drainage without treatment.

Table 2: Physico-chemical characteristics of cardboard industrial effluent (average of the data for the period of 12 months)

S.No.	Parameters	Values (Mean ± Standard Deviation)
1	Temperature	20.6 - 37.6
2	pH	6.61 ± 0.3
3	Conductivity	4.24 ± 0.5

4	TDS	2.02 ± 0.2
5	Salinity	1.35 ± 0.5
6	Colour (Pt-Co units)	1005.53 ± 986.6
7	Turbidity (NTU)	31.8 ± 22.11
8	BOD (mg/L)	25.21 ± 5.8
9	COD (mg/L)	4500.0 ± 1299.7

Analysis of *physicochemical parameters is not sufficient to decide the safe quality of the effluent*. Therefore, in the present *work mutagenicity of the effluent was also assessed* using *Salmonella typhimurium* strain TA 98 and TA 100. Results of mutagenicity assay (Figure 1) revealed that effluent is mutagenic with *Salmonella typhimurium* TA 98 and TA 100 strains which shows the presence of frameshift and basepair mutagens respectively. Further,a significant increase in mutagenicity was observed on adding S9 mix, possibly due to the activation of mutagens by the metabolic action of hepatic fraction. This reveals that hepatic fraction unable to detoxify the action of mutagens. Therefore, these *industries cannot be considered as ecofriendly.*

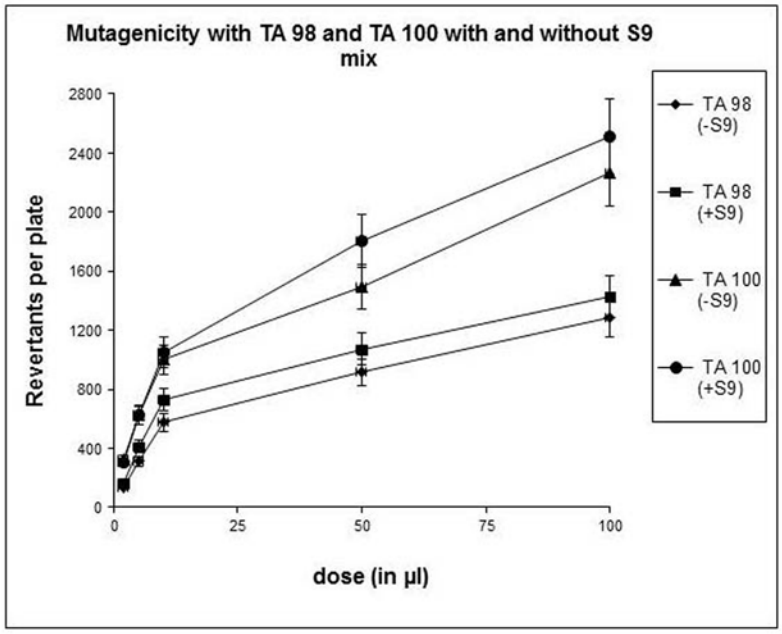

Figure 1: Frameshift and basepair mutagenicity revealed by *Salmonella typhimurium* TA 98 and TA 100 strains in the presence (+S9) and absence (-S9) of S9 mix.

Cardboard industrial effluent was treated in aerobic condition with three white rot fungi i.e. *Phanerochaetechrysosporium, Pleurotus florida*and *Pleurotus citrinopileatus*because *effluent was found to be acidic and fungi can be adapted well in acidic environment.* All these strains were screened for the production of cellulolytic activities and results of average and standard deviation of three replicates are shown in Table 3.Cellulase production by

the selected strains was found to increase with respect to time. However, all strains were not significantly different for relative enzyme activity.

Table 3: Growth of fungal strains on PDA and CMC media and relative enzyme activity of cellulase on CMC medium only are shown.

Relative enzyme activity (I_{CMC}) in 48 h					
S. No.	**Name of Fungal Cultures**	**Growth on PDA (in cm) after 48 hrs.**	**Growth on CMC medium (in cm) after 48 hrs.**	**Clearing Zone diameter (in cm)**	**Relative enzyme activity (I_{CMC}) on 48hrs.**
1	*P.chrysosporium*	1.9 ± 0.1	0.1 ± 0.05	0.33 ± 0.0	3.3 ± 1.15
2	*P. florida*	0.2 ± 0.05	0.1 ± 0.05	0 ± 0	0 ± 0
3	*P.citrinopileatus*	0.2 ± 0.05	0.2 ± 0.05	0.11 ± 0.05	0.57 ± 0.05
Relative enzyme activity (I_{CMC}) in 72 h					
1	*P.chrysosporium*	2.53 ± 0.05	0.37 ± 0.05	1.67 ± 0.05	4.6 ± 0.8
2	*P. florida*	2.73 ± 0.05	0.3 ± 0.05	0.9 ± 0.1	2.7 ± 0.25
3	*P.citrinopileatus*	2.83 ± 0.05	0.36 ± 0.05	1.13 ± 0.2	3.09 ± 0.14
Relative enzyme activity (I_{CMC}) in 96 h					
1	*P.chrysosporium*	2.96 ± 0.05	0.63 ± 0.1	3.13 ± 0.05	5.06 ± 0.98
2	*P. florida*	3.53 ± 0.1	0.86 ± 0.15	2.8 ± 0.2	3.23 ± 0.15
3	*P.citrinopileatus*	3.43 ± 0.05	0.93 ± 0.05	3.13 ± 0.2	3.35 ± 0.1

$p>0.05$ means of relative enzyme activity are not significant for fungi

$p<0.05$ relative enzyme activity significantly increase with respect to time.

Before employing any treatment techniques, it is pertinent to note that the main cause failure of implementing the developed treatment technology at large scale is – lack of set up of exactly the same conditions which are in the field. In most of the cases effluent has treatedin artificial conditions, such as in diluted form, by supplementing nutrients, setting of pH, temperature *etc*. and by choosing a synthetic compound only.The most important thing to be considered is not the compounds formed by microbial treatment, but the effluent must possess all the parameters

withinthe discharging limits including toxicity. Hence, the present study focused on assessing the effect of fungal and activated sludge treatment *not only on physicochemical parameters but also on mutagenicity of cardboard industrial effluent in nutrient amended (denoted as "+") and non-amended conditions (denoted as "-").*In this study, effluent was neither diluted nor sterilized. As mentioned earlier, these were treated directly in raw form as obtained from industriesin order to decide the suitability and adaptability of fungal strain in natural condition.

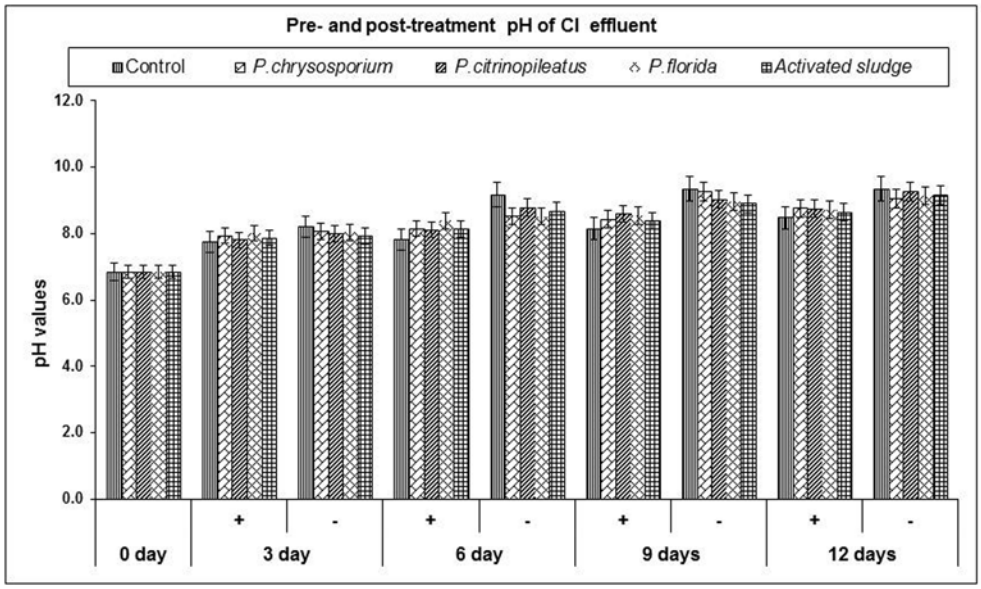

Figure 2: The change in pH of cardboard industrial effluent by fungi and activated sludge in nutrient amended (+) and non-amended (-) condition.

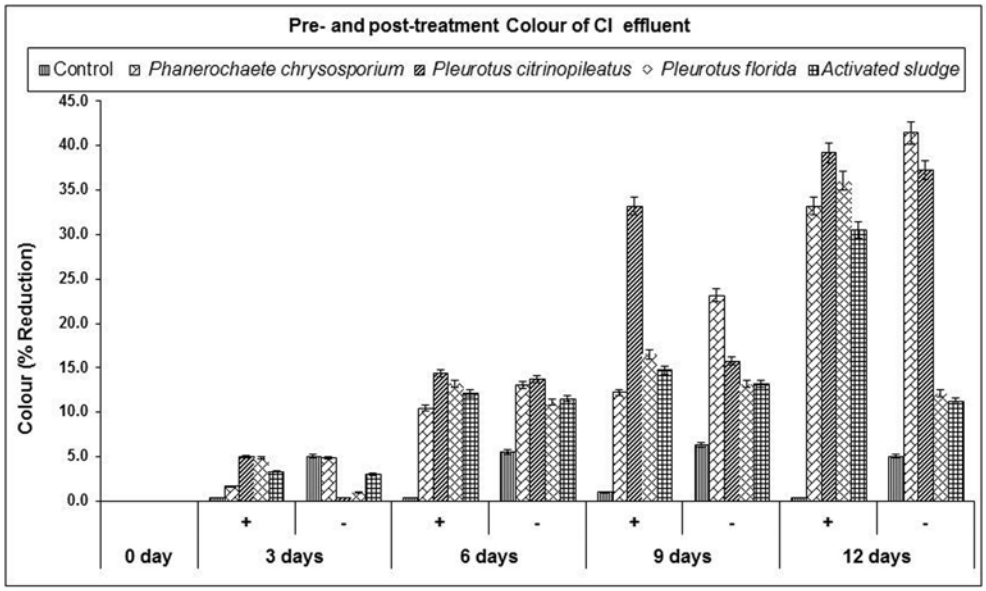

Figure 3: The change in colour (% reduction) of cardboard industrial effluent by fungi and activated sludge in nutrient amended (+) and non-amended (-) condition.
"*" $p<0.05$ control vs. all treatments
"$" $p<0.05$ 0 day vs. 12^{th} day

The pH of the effluent was found to be slightly acidic (6.8) initially (Table 4), however, after fungal treatment it was found to be shifted towards the basic scale. The change in pH was not significant among all treatments (Figure 2) in both nutrient amended and non-amended condition. Similarly, all treatments were not found to be very effective in reducing the colourin nutrient supplemented and non-supplemented condition from their initial value i.e. 12404 Pt-Co units (Table 4). In nutrient non-amended condition, the maximum colour reduction (41.5%) was observed with the *Phanerocheatechrysosporium*($p<0.05$) in 12 days retention time (Figure 3).However, *Pleurotus citrinopileatus* reduced the colour in 9 days in nutrient amended condition. The brown colour became lighter, but not completely disappeared after the treatment.

Table 4: Physico-chemical parameters and mutagenicity ratio of cardboard industrial effluent on initial day

Physico-chemical parameters			Mutagenicity Ratio (Ames test)				
pH	COD	Colour (Pt-Co units)	Dose	TA 98		TA 100	
6.8	7223.3 mg/L	12404.1	(in ul)	-S9	+S9	-S9	+S9
			2	0 (-)	0 (-)	1.2 (-)	1.2 (-)
			5	0 (-)	0 (-)	3.3 (+)	2.4 (+)
			10	0 (-)	0 (-)	5.1 (+)	3.1 (+)
			50	1 (-)	1 (-)	7.2 (+)	5.5 (+)
			100	1(-)	1 (-)	14.2 (+)	8.9 (+)

On initial day, COD of the effluent was 7223.3 mg/l (Table 4). In nutrient amended and non-amended condition, COD was found to decrease with all fungi in 6 to 12 days ($p<0.05$). All treatments were found to be effective in reducing the COD in both conditions ($p<0.05$). The most significant COD reduction was observed with activated sludge (93.9%)in 12 days without supplementation of nutrients, which is followed by *Pleurotus citrinopileatus*(93.4%), *Pleurotus florida*(68.6%)and *Phanerochaetechrysosporium*(59.6%)(Figure 4). In contrast to this, *Phanerochaetechrysosporium*(93.9%) and *Pleurotus florida*(87.8%) can effectively reduce the COD in nutrient amended condition. This shows that same level of reduction in COD is achieved by activated sludge and *Phanerchaetechrysosporium* in absence and presence of nutrients respectively.

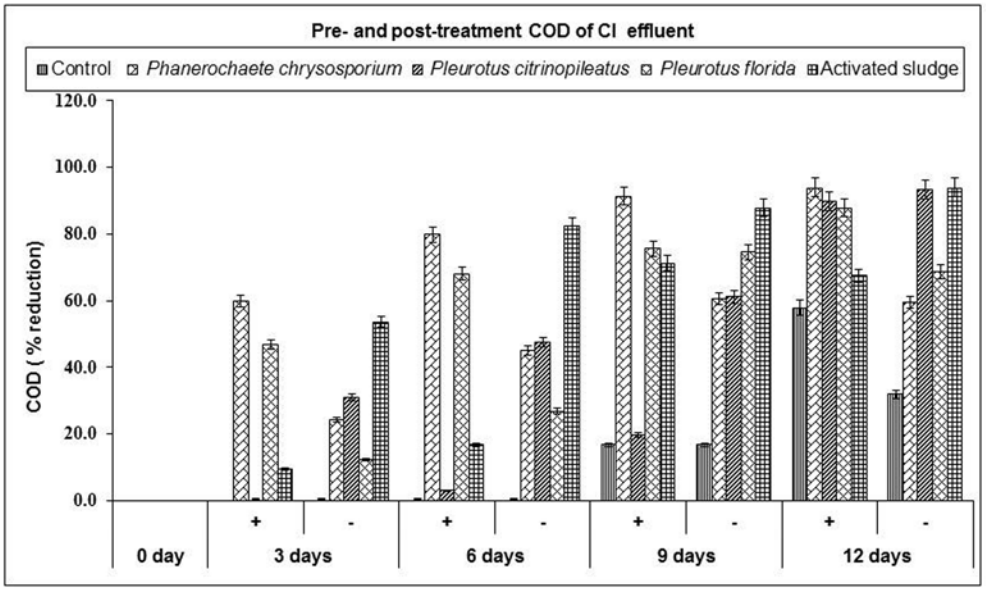

Figure 4: The change in COD (% reduction) of cardboard industrial effluent by fungi and activated sludge in nutrient amended (+) and non-amended (-) condition.
"*" $p<0.05$ control vs. all treatments
"$" $p<0.05$ 0 day vs. 12^{th} day

Effluent employed in this study did not possess frameshift mutagens as indicated by its mutagenicity ratio (Table 4). Further, it did not show any type of mutagenicity in the presence of hepatic fraction S9 mix with *S.typhimurium* TA 98. In contrast to this, effluent was found to be mutagenic with *S.typhimurium* TA 100 at all high dose levels, which revealed the presence of basepair mutagens (Table 4).

On non-supplementation of effluent with nutrient, basepair mutagenicity was found to be decreased with respect to initial day. After 6 days, *Pleurotus florida* was found to be most effective in reducing the mutagenicity while activated sludge was not able to reduce mutagenicity compared to control. Further, this mutagenicity did not increase on adding S9 mix ($p<0.05$) as revealed by mutagenicity ratio (Table 5). This shows that basepair mutagens produce some compounds by fungal degradation which is not mutagenic to eukaryotic system and liver enzymes are not able to detoxify them. Similarly, *Pleurotus florida* was found to be most effective in reducing the mutagenicity in nutrient amended condition which is followed by *Phanerochaetechrysosporium* and *Pleurotus citrinopileatus*. Again, mutagenicity was not found to be increased by adding S9 mix because mutagenicity ratio was found to be below 2($p<0.05$) (Table 5) which reveals that liver fraction is effective in reducing the mutagenicity.Therefore, "Fungi are able to reduce the mutagenicity" is found in agreement with the previous reports [6, 20].

Table 5: Mutagenicity ratio of the cardboard industrial effluent treated with fungi and activated sludge in nutrient amended and non-amended conditions in the presence (+ S9) and absence (-S9) of S9 mix.

		Nutrient amended							
	Sample withdrawl→	3 day		6 day		9 day		12 day	
S.No.	Treatment	- S9	+ S9	- S9	+ S9	- S9	+ S9	- S9	+ S9
1	Control	6.6 (+)	3.8 (+)	5.9 (+)	2.7 (+)	5.2 (+)	3 (+)	7.3 (+)	3.1 (+)
2	*P.chrysosporium*	6.7 (+)	2.8 (+)	2.6 (+)	1 (-)	1 (-)	1 (-)	1 (-)	1 (-)
3	*Pleurotus citrinopileatus*	5.6 (+)	2.5 (+)	3.1 (+)	1 (-)	1 (-)	1 (-)	1 (-)	1 (-)
4	*Pleurotus florida*	4.0 (+)	2.6 (+)	2.8 (+)	1 (-)	1 (-)	1 (-)	1 (-)	1 (-)
5	Activated sludge	7.2 (+)	2.9 (+)	5 (+)	2.7 (+)	1.1 (-)	1 (-)	2.2 (+)	1 (-)
		Nutrient non-amended							
	Sample withdrawl→	3 day		6 day		9 day		12 day	
S.No.	Treatment	- S9	+ S9	- S9	+ S9	- S9	+ S9	- S9	+ S9
1	Control	16.2 (+)	3.2 (+)	13 (+)	3.4 (+)	6.3 (+)	2.4 (+)	6.6 (+)	2.6 (+)
2	*P.chrysosporium*	4.5 (+)	1	2.8 (+)	1 (-)	1 (-)	1 (-)	1 (-)	1 (-)
3	*Pleurotus citrinopileatus*	6.4 (+)	2 (+)	2.4 (+)	0.2 (-)	1 (-)	1 (-)	0.08 (-)	1 (-)
4	*Pleurotus florida*	1.2	1 (-)	0.7 (-)	1 (-)	1 (-)	1 (-)	1.1 (-)	1 (-)
5	Activated sludge	4.6 (+)	1.6 (-)	3.2 (+)	2.1 (+)	2.4 (+)	0.8 (-)	2 (+)	0.6 (-)

All fungal strains reduced the colour of the effluent but cannot completely remove it. The maximum color reduction was obtained with *P. chrysosporium* and *Pleurotus citrinopileatus* in nutrient amended and non-amended condition respectively. These strains shifted the pH towards basic scale. *Pleurotus florida* was found to be the most important strain in this study *due to its ability to reduce mutagenicity irrespective of nutrient requirement*. However, it needs the supply of nutrient for reducing COD. This is in contrast to the previous reports on the same fungi in which *P.florida* was found to be most effective in reducing all parameters of handmade paper industrial effluent[6]. It is clear from aforesaid description that a single fungus is not effective in reducing all the parameters of effluent. However, these can treat the effluent effectively in natural unmodified conditions. Therefore, there is a need to *develop a consortium of fungi for complete treatment and reducing the physico-chemical parameters as well as mutagenicity.*

4. Conclusion

Cardboard industrial effluent can be treated with fungi in the presence or absence of nutrient. The efficacy of the process depends upon the strain selected for the treatment. In this case, none of the strain was found to be very effective in reducing all the parameters. Therefore, a consortium by using different fungi can be developed for reducing not only physicochemical parameters but also mutagenicity.

5. Acknowledgement

Financial assistance provided by Department of Science and Technology, Jaipur is highly acknowledged.

6. References

1. Kulshreshtha, S., Mathur, N., Bhatnagar, P., Jain B.L.: Bioremediation of industrial wastes through mushroom cultivation. J.Environ. Biol. 31, 441-444 (2010)
2. Gado, I.H., Ouiminga, S.K., Daho, T., Yonli, A.H., Sougoti, M., Koulidiati, J.: Characterization of briquettes coming from compaction of paper and cardboard waste at low and medium pressures. Waste Biomass Valorization. 5, 725-731 (2014)
3. Bhuwal, A.K., Singh, G., Aggarwal, N.K., Goyal, V., Yadav, A.: Isolation and screening of polyhydroxyalkanoates producing bacteria from pulp, paper, and cardboard industry wastes. International Journal of Biomaterials (2013). doi: 10.1155/2013/752821
4. Amat, A.M., Arques, A., Miranda, M.A., López, F.: Use of ozone and/or UV in the treatment of effluents from board paper industry. Chemosphere. 60:1111- 1117 (2005)
5. Punzi, M., Nilsson, F., Anbalagan, A., Svensson, B.M., Jönsson, K., Mattiasson, Bo, Jonstrup, M.: Combined anaerobic–ozonation process for treatment of textile wastewater: Removal of acute toxicity and mutagenicity. J. Hazard. Mater. 292, 52–6015 (2015)
6. Kulshreshtha, S., Mathur N., Bhatnagar, P.: Aerobic treatment of handmade paper industrial effluents by white rot fungi. Journal of Bioremediation and Biodegradation, (2012). doi: 10.4172/2155-6199.1000151
7. Kulshreshtha, S., Mathur N., Bhatnagar, P.: Seasonal analysis of Sanganer handmade paper and cardboard industries effluent. Indian Journal of Environment and Ecoplanning. 15, 373-378 (2008)
8. CPCB.: Minimum National Standards (MINAS) for large pulp and paper mills with chemical recovery. , (2000)
9. APHA: Standard methods for the examination of water and waste water. APHA, AWWA, WPCF, Washington DC, USA (1995)
10. Maron, D.M., Ames, B.N.: Revised methods for the Salmonella mutagenicity test. Mut. Res. 113, 173-215 (1983)
11. Prival, M.J.: Mitchell Analysis of a method for testing azo dyes for mutagenic activity in *Salmonella typhimurium* in the presence of flavinmononuleotide and hamster liver S9. Mut. Res. 97, 103-116 (1982)
12. Peciulyte, D.: Isolation of cellulolytic fungi from waste paper gradual recycling materials. Ekologija. 53, 11-18 (2007)
13. Teather R.M., Wood P.J.: Use of congo red-polysacharide interactions in enumeration and characterization of celluloytic bacteria in the bovine rumen. Appl. Environm. Microbol. 43, 777-780 (1982)
14. Libra, J.A., Borchert, M., Banit, S.: Competition Strategies for the decolorization of a textile-reactive dye with the white-rot fungi *Trametesversicolor* under nonsterile conditions. Biotechnol. Bioeng. 82, 736-744 (2003)
15. Belsare, D.K., Prasad, D.Y.: Decolorization of effluent from the bagasse based pulp mills by white-rot fungus, *Schizophyllum commune*. Appl. Microbiol. Biotechnol. 28, 301-304 (1988)
16. Wu, J., Xiao, Y.Z., Yu, H.Q.: Degradation of lignin in pulp mill wastewaters by white-rot fungi on biofilm. Bioresource Technol. 96, 1357-1363 (2005)
17. Pant, D., Singh, A., Satyawali, Y., Gupta, R.K.: Effect of carbon and nitrogen source amendment on synthetic dyes decolourizing efficiency of white-rot fungus, *Phanerochaetechrysosporium*. J. Envrion. Biol. 29, 79-84 (2008)
18. Martin, C., Manzanares, P.: A study of the decolourization of straw soda pulping effluents by *Trametesversicolor*. Bioresource Technol. 47, 209-214 (1994)
19. Mortelmans, K., Zeiger, E.: The Ames *Salmonella* / microsome mutagenicity assay. Mutat. Res. 455, 29-60 (2000)
20. Malachová, K., Pavlícková, Z., Novotný, C., Svobodová, K., Lednická, D. and Musílková, E. Reduction in the mutagenicity of synthetic dyes by successive treatment with activated sludge and the ligninolytic fungus, *Irpexlacteus*. Environ. Mol. Mutagen. (2006). doi: 10.1002/em.20224