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PHYSIOCHEMICAL AND MICROBIAL STUDIES OF PAPER MILL EFFLUENT, RAIPUR (CHHATTISGARH), INDIA

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Abstract. The present study report physiochemical properties of paper mill effluent and fungal diversity present in effluent. The fungi were further studied for production of organic waste degrading enzymes. Water quality analyzer, turbiditymeter and spectroquant nova 60 and titration method were used to physiochemical properties of paper mill effluent. Fungi were isolated from effluent in agar plate. Organic waste degrading amylolytic and cellulolytic enzyme produce by fungi were recorded in agar plate. The mean value of different physiochemical properties were found as follows: pH 7.50, EC 4.26 mS, TSS 0.28 mg/L, TDS 436.4 mg/L, Turbidity 145.26 NTU, DO 4.48 mg/l, BOD 48.26 mg/L, COD 54.14 mg/L, fluoride 1.15 mg/L, lead 1.78 mg/L and iron 0.51 mg/L. *Aspergillus niger, A. fumigates* and *Penicillium oxalicum* were found as dominant species. Fungi isolated from paper mill effluent produced enzymes to degrade the organic waste substances.

Keywords: biodiversity of fungi, enzymatic potential fungi, paper mill effluent, physiochemical properties.

Introduction

The paper mills uses water and lignocelluloses woods for making of paper products these materials release large quantity of effluent containing fibers, chemicals, synthetic dyes and wood [SUNTIO *et al*., 1988; LACORTE *et al*., 2003].

The process in paper mill includes wood preparation, pulping, pulp washing, screening, washing, bleaching and coating operation plays major role in releasing of waste water [KUZHALI *et al*., 2012].

The Raipur city is sandwich between two industrial areas, first includes Urla, Siltara, Birgaon, Bhanpuri and Godwara and second Bhilai steel plant. The city receives pollutants from both sides according to the wind direction.

Due to high mineral viability most of industries are coal based iron factory but more than 10 paper mills are also working in city.

The available lignolytic and cellulolytic crops around Raipur are rice, wheat, sugarcane *etc*. for establishment of paper mills.

The enzymes may ultimately provide a better answer to solve the problems associated with cellulosic waste disposal releases from various sources [ROY *et al*., 1990].

The study of properties of industrial effluent in Chhattisgarh is poorly known so the present work aiming to investigate physical and chemical properties of waste water and to find out biodiversity of fungi adopted for degrading organic waste using production of enzymes.

Material and methods

Study area and sample collection

In the present investigation effluent was collected from Paper mill industry situated at Urla industrial area Raipur, it has distance of 06 km from research centre.

The effluent is running open about 150–200 meter releasing from industry.

Waste water sample was collected two months in winter season (December 2011 and January 2012) where maximum cold observed in study area, two months in summer season (April and May 2012) where maximum temperature observed and two month in rainy (July and August 2012) where the maximum precipitation observed.

The effluent has been collected in plastic container washed with detergent and doubled distilled water.

The sampling was done between the duration of 05 PM to 07 PM.

Study of physiochemical parameters

The observation of pH, temperature, TDS and conductivity were taken from Water Quality Analyzer PE 138 (Develop by ELICO India).

Effluent was filter by Whatman Filter Paper No. 1 then separate electrode were used for detection of pH, temperature and conductivity using water quality analyzer. Turbidity was measured in nephelometric turbidity units (NTUs) using Turbiditimeter TN–100 (Develop by Eutech Instruments).

Dissolve oxygen (DO) was measured by titration method using Sodium thiosulfate, Manganous hydroxide and Alkaline iodine azide.

Titration was done by standard sodium thiosulfate solution using starch as indicator.

Chemical oxygen demand (COD) taken also by titration method using Potassium dichromate as chemical oxidant for organic matter present in waste water.

Titration was done by sodium thiosulfate solution starch as indicator. Biological oxygen demand (BOD) was measured as BOD_3.

Fluoride, Iron and Lead were measure by Spectroquant Nova60 (develop by Merk Germany) as mg/liter [THOMAS *et al.*, 2011].

Isolation and identification of fungi

Fungi were isolate from paper mill effluent on Potato dextrose agar (PDA) plates under aseptic condition.

Five PDA plates were taken for each sample and serially diluted 10^{-1} to 10^{-4} concentration of sample were poured. Pure cultures of fungal slants were maintained at 4°C on freezer.

The identification of fungi was initially done at School of Studies in Biotechnology, Pt. Ravishankar Shukla University Raipur (C.G.) with available literature, further culture were sent to National centre for Fungal Taxonomy (NCFT) New Delhi for morphological identification of fungi.

Enzyme activity test

The methodology of amylolytic and cellulolytic activity of fungi was little modified from [HANKIN and ANAGNOSTATIS, 1975]. The isolates fungi were tested for amylolytic activity on starch agar medium (SAM) plate.

Fungi were incubate to 7 days on 26±1°C and after this Iodine solution (3% KI and 0.3% I) was flooded to the medium.

A yellow zone around the fungal colony indicated amylolytic activity of fungi.

Similarly cellulolytic activities of fungi were studied on yeast peptone medium containing 0.5% Carboxymethylcellulose (CMC). After 7 day of incubation of fungi Iodine solution (3% KI and 0.3% I) was flooded to the medium. A hollow zone appearance around the fungal colony indicates Cellulase activity.

Results and Discussion

Physiochemical properties of paper mill effluent

During each sampling color of effluent was observed blackish and unpleasant smell was found.

The minimum temperature was 22°C in January and maximum was recorded 30°C in the month of May.

pH of the sample effluents was recorded from 6.87 minimum (December) to 8.40 maximum (January).

Electric conductivity of effluent was observed varying throughout the year.

It was 1.70mS (January) minimum and 9.10 mS (August) as maximum. 1.03 to 1.92 mS electric conductivity waste recorded from three different paper effluent collected from Lalkuan, Nainital India [MALAVIYA and RATHOR, 2007], but in present investigation mean of EC was found 4.26 mS.

Turbidity of paper effluent from present observation was found varying according to the different season its mean value was recorded 145.26 NTU.

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Similarly, 51.00, 121.00 and 259.00 NTU turbidity were recorded from three different sample effluents of paper mill [EMEKA *et al*., 2011].

Dissolved oxygen was found average of 4.48 mg/l. Mean BOD_3 of present work was found 48.28 mg/L.

The BOD was found minimum 0.48 mg/L to 554 mg/L maximum by some previous investigators [MALAVIYA and RATHORE, 2007; EMEKA *et al*., 2011; MISHRA *et al*., 2012].

Mean of chemical oxygen demand (COD) of present work was recorded 54.14 mg/l with highest of 92.36 mg/l in the month of May while minimum was observed 16.93 mg/l (August). Physiochemical properties of paper mill effluent are presented in Table 1.

Table 1.

Physiochemical Properties of Paper mill effluent from Dec. 2011 to Aug. 2012

No.	Physiochemical Parameters	Units	Winter		Summer		Rainy		Mean (6 months)
			December (2011)	January (2012)	April (2012)	May (2012)	July (2012)	August (2012)	
1	**Temperature**	°C	23	22	28	30	25	24	25.33
2	**pH**	pH	6.87	8.40	7.74	6.92	7.43	7.69	7.50
3	**EC**	mS/cm	7.90	1.70	2.54	2.11	2.20	9.10	4.26
4	**TSS**	mg/l	0.26	0.11	0.36	0.22	0.41	0.37	0.28
5	**TDS**	mg/l	409.5	888.9	630.0	528.0	101.0	223.0	436.4
6	**Turbidity**	NTU	105.1	126	296	204	47.3	93.2	145.26
7	**DO**	mg/l	3.21	5.60	3.61	4.11	6.12	4.23	04.48
8	**BOD**	mg/l	48.48	61.8	51.2	72.36	31.60	21.12	48.26
9	**COD**	mg/l	56.00	92.36	65.76	69.12	24.68	16.93	54.14

The mean of Pb and Fe were found 1.78 and 0.51 mg/L in present work.

The lead was found maximum (2.65 mg/l) in the month of April while iron was found maximum 0.93 mg/L in January.

Fluoride content was found maximum of 1.24 mg/L (July) and its mean value was 1.15 mg/L. 0.07 to 1.21 mg/L fluoride recorded from paper mill waste [SMITH and MOOLLAN, 2004; MEENAMBAL *et al.*, 2011].

Data of Iron, fluoride and lead from December 2011 to August 2012 was shown in the figure 1.

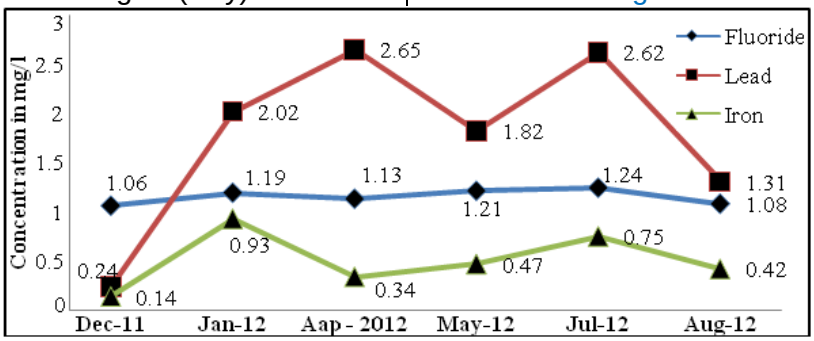

Figure 1. Fluoride, Lead and Iron content of Paper mill effluent

Finding of present work is compare with the National standard of Central pollution control board (CPCB) New Delhi, based on The Environment (Protection) Rules, 1986. Suspended solids, pH, COD, fluoride and iron were found below standard while BOD and Lead content of paper mill effluent was found higher than standard.

Biodiversity of fungi

Twelve fungal species (21 colonies) were isolated from present work of 6 six month. Maximum fungi were found in winter followed by rainy and summer. Fungi with 6 species (7 colonies) were observed as maximum in the month of December while least fungus was recorded in the month of April with only one species (1 colony). The *Aspergillus fumigates, A. niger* and *Penicillium oxalicum* were found as dominant species, *Aspergillus restricted, Cladosporium cladosporides* and *Penicillium chrysogenum* were recorded as moderate dominant while *Aspergillus*

paraciticus, Emericella nidulans, Mucor cymosus, Penicillium citrinum, Phoma exigua and Trichoderma viride were found as least dominant in paper mill effluent (Table 2).

Table 2.

Seasonal and month wise distribution of fungi isolates from paper mill effluent

No.	Fungal species	Division	Occurrence						%F
			Winter		Summer		Rainy		
			Dec	Jan	Apr	May	Jul	Aug	
1	*Acremonium restricted*	Deuteromycotina	+	+	–	–	–	–	9.52
2	*Aspergillus fumigates*	Deuteromycotina	+	–	–	–	–	+	14.28
3	*Aspergillus niger*	Deuteromycotina	+	–	+	–	+	–	14.28
4	*Aspergillus parasiticus*	Deuteromycotina	–	–	–	–	+	–	4.76
5	*Cladosporium cladosporides*	Deuteromycotina	+	+	–	–	–	–	9.52
6	*Emericella nidulans*	Ascomycotina	+	–	–	–	–	–	4.76
7	*Mucor cymosus*	Zygomycotina	–	+	–	–	–	–	4.76
8	*Penicillium citrinum*	Deuteromycotina	–	–	–	–	–	+	4.76
9	*Penicillium chrysogenum*	Deuteromycotina	–	–	–	+	–	–	9.52
10	*Penicillium oxalicum*	Deuteromycotina	+	+	–	–	–	+	14.28
11	*Phoma exigua*	Deuteromycotina	–	–	–	–	–	+	4.76
12	*Tricoderma viride*	Deuteromycotina	–	+	–	–	–	–	4.76

The biodiversity of fungi in industrial waste habitat was studied worldwide because of its great potency toward production of metabolites and accumulation of pollutants. Genus of *Aspergillus,* was recorded as dominant followed by *Penicillium* and *Trichoderma* in previous study biodiversity of fungi in paper mill effluent [SENTHILKUMAR and PANNEERSELVAM, 2010; BAJWA *et al.,* 2010].

Amylolytic and cellulolytic activity of fungi

Among twelve fungal species six were found amylolytic potential these species belongs to *Aspergillus restricted, A. fumigates*, *Cladosporium cladosporides, Penicillium citrinum, P. chrysogenum* and *P. oxalicum.*

Studied with cellulolytic activity seven out of twelve species observed as cellulolytic positive. *Aspergillus restricted, A. fumigates*, *Cladosporium cladosporides, Penicillium citrinum, P. chrysogenum* and *P. oxalicum* were found both amylolytic and cellulolytic potential species, while *Aspergillus niger* showed only Cellulase activity test. The rest fungal species including, *Aspergillus parasiticus, Emericella nidulans, Mucor cymosus, Phoma exigua* and *Trichoderma viride* observed neither amylase producing not Cellulase producing (Table 3).

Table 3.

Enzymatic properties of some fungal species isolates from paper mill effluent

No.	Fungi	Amylolytic activity	Cellulolytic activity
1	*Acremonium restricted*	+	+++
2	*Aspergillus fumigates*	++	++
3	*Aspergillus niger*	–	++
4	*Aspergillus parasiticus*	–	–
5	*Cladosporium cladosporides*	++	+++
6	*Emericella nidulans*	–	–
7	*Mucor cymosus*	–	–
8	*Penicillium citrinum*	+	+++
9	*Penicillium chrysogenum*	+++	+++
10	*Penicillium oxalicum*	+++	+++
11	*Phoma exigua*	–	–
12	*Tricoderma viride*	–	–

Findings of present investigation showed half of the total species (50%) were found as amylolytic potential while more than half (58.33%) as celluloytic potential. The results of present work indicate effect of habitat on enzymatic potentiality of fungi.

Fungus isolated from all three season was *Aspergillus niger* and some fungi isolated from two different season included *Aspergillus fumigatus* and *Penicillium oxalicum*.

These fungi have been shown enzymatic potential to degrade organic source.

While fungi isolated from only one season which shown enzymatic potential were only 44.44 percent (Figure 2).

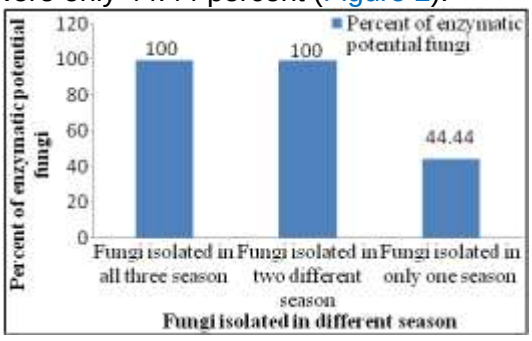

Figure 2. Enzymatic potentiality of fungi isolated from different season

So that, from figure 2 it was concluded that fungi present more duration in paper mill effluent may adopted on it and produced enzyme to degrade organic waste released from industry.

Habitat effects the biodiversity of organism [FAHRIG, 2003], similarly investigate effect of geographic location and habitat also effect distribution and occurrence of fungi [VANNINEN, 1996].

Some amylolytic potential *Aspergillus* sp., *A. fumigatus* and *Penicillium* sp. were isolated from agriculture waste [ADENIRAN and ABIOSE, 2009; JAVED *et al.*, 2011].

Conclusion

During present investigation significant increase in level of some parameter was obtained while some are under the National standard.

The waste tolerating diversity of fungi was obtained from effluent containing potency to produce significant enzyme to degrade the waste substrate.

These findings indicate the effect of habitat on biodiversity and nature of fungi.

Fungi belonging to the genus of *Penicillium* have been shown maximum potency to produce enzyme followed by genus of *Aspergillus* which will useful to dispose the cellulolytic waste released from various sources.

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References

1. Adeniran, A.H.; Abiose, S.H.; Amylolytic potentiality of fungi isolated from some Nigerian agricultural wastes, *African Journal of Biotechnology* **2009,** *8*(4):667–672.
2. Bajwa, R.; Khokhar, I.; Mukhtar, I.; Mushtaq, S.; Isolation and Identification of Filamentous Fungi from Different Industrial Effluents, *Plant Products Research Journal*. **2010**, *4*:32–35.
3. Emeka, I.C.; Ihediohamma, E.E.; Linus, N.; Ndubuisi, U.C.; Ebele, A.G.; Physicochemical dynamics of the impact of paper mill effluents on Owerrinta River, eastern Nigeria, *Journal of Environmental Chemistry and Ecotoxicology.* **2011**, *3*(11):298–303.
4. Fahrig, L.; Effects of Habitat Fragmentation on Biodiversity. *Annual Review of Ecology Evolution, and Systematics*. **2003***, 34*:487–515.
5. Hankin, L.; Anagnostatis, S.L.; The Use of Solid Media for Detection of Enzyme Production by Fungi. *Mycologia*. **1975***, 67*(3):597–607.
6. Javed, M.M.; Ikram–Ul–Haq; Mariyam, I.; Latif, F.; Distribution of Cellulolytic–Thermophilic Fungi on various Substrates and Geographic Locations in *Pakistan. Pak. J. Bot.* **2011***, 43*(5):2621–2625.
7. Kuzhali, S.S.; Manikandan, N.; Kumuthakalavalli, R., Physico chemical and biological parameters of paper industry effluent. *J. Nat. Prod. Plant Resour.* **2012**. *2*(3):445–448.
8. Lacorte, S.; Latorre, A.; Barcelo, D.; Rigol, A.; Malmqvist, A.T.; Welander;

Organic compounds in paper–mill process water and effluents. *Trends in Analytical Chemistry*. **2003**, *22*(10):725–737.
9. Meenambal, M.; Pugazhendy, K.; Vasantharaja, C.; Venkatesan, S.; Effect of paper mill effluent on the ground water quality in and around Adikaripatti area of Salem, Tamilnadu. *Golden Resarch Thoughts*. **2011**, *1*(3):1–4.
10. Mishra, S.; Mohanty, M.; Pradhan, C.; Patra, H.K.; Das, R.; Sahoo, S.; Physico–chemical assessment of paper mill effluent and its heavy metal remediation using aquatic macrophytes–a case study at JK Paper mill, Rayagada, India. *Environ. Monit. Assess.* **2012**, doi 10.1007/s10661–012–2873–9.
11. Roy, S,K.; Dey, S.K.; Raha, S.K.; Chakraberty, S.L.; Purification and properties of an extracellular endoglueanase from *Mycoliophthora thermophilia*, D–14 (ATCC 48104). *J. Microbiol*. **1990**, *136*:1967–1971.
12. Ryu, D.D.; Mandels, M.; Cellulase: Biosynthesis and Applications. *Enzyme Microbiol. Technol.* **1980**, *2*:92–102.
13. Senthilkumar, G.; Panneerselvam, A.; Biodiversity of Microbes in Paper Mill Effluent. *Pollution research paper*. **2010**, *29*(1):33–36.
14. Smith, A.; Moollon, R.; An overview of the fluoride level in waste water in the city of Cape Town. In: *Proceeding of the 2004 water institute of southern Africa (WISA)* Biennial conference, **2004**, pp. 1516–1521.
15. Suntio, L.R.; Shiu, W.Y.; Mackay, D., A: Review of the nature and properties of chemicals present in pulp mill effluents, *Chemosphere*. **1988** *17*(7):1249–1290.
16. Thomas, D.R.; Sunil, B.; Latha, C.; Physico–Chemical Analysis of Well Water at Eloor Industrial Area–Seasonal Study. *Current World Environment*. **2011**. *6*(2):259–264.

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