PROJECT REPORT

A SYNERGISTIC AND ECONOMICAL APPROACH FOR TREATMENT OF PULP AND PAPER MILL EFFLUENT SYSTEM USING MICROBES AND FLY–ASH NANOPARTICLES TO ACHIEVE MINIMUM/ ZERO WASTE DISCHARGE

Submitted by

Chemical Recovery & Biorefinery Division



Central Pulp & Paper Research Institute Saharanpur, (U.P.), India

&

Department of Biotechnology



Amity University Uttar Pradesh, Sector-125, Noida, Uttar Pradesh

Project Particulars

- 1. Name of the funding agency: Ministry of Commerce and Industry
- 2. Reference No. provided by funding agency:
- Title of the Project: A synergistic and economical approach for treatment of pulp and paper mill effluent system using microbes and fly-ash nanoparticles to achieve minimum/ zero waste discharge
- 4. Name of the PI and address: (i) Prof.(Dr) Rachana Singh Amity University Uttar Pradesh, Sector-125, Noida, Uttar Pradesh (ii) Dr Ashwani Kumar Dixit Central Pulp and Paper Research Institute, Saharanpur
- 5. Name of the Co-PI(S) and address: (i) Dr Surbhi Sinha
 Amity University Uttar Pradesh, Sector-125, Noida, Uttar Pradesh
 (ii) Mr Kumar Anupam
 Central Pulp and Paper Research Institute, Saharanpur
- 6. Date of sanction of project:
- 7. Date of commencement of project: 09.01.2019
- 8. Total Cost of the Project: Rs 42.75 Lakhs
- 9. Total duration of project in months: 30 months

S.No	Approved Objectives	Progress against each	Any shortfall/
		objective	over achievement/
			deviation
1.	Characterisation of paper mill effluent	Achieved	Nil
2.	Screening and identification of lignolytic bacteria from effluent	Achieved	Nil
3.	Collection and preparation of fly ash nanoparticles	Achieved	Nil
4.	Optimisation of the experimental parameters for synergistic effects of bacteria as well as fly ash nanoparticles together for colour removal and other pollutants	Achieved	Nil
5.	Fabrication and optimization of the treatment system for paper effluent at bench scale	Achieved	Nil
6.	Assessment of toxicity of the treated water by cytogenotoxicity	Achieved	Nil
7.	Reportpreparationanddissemination of findings	Achieved	Nil

10. Table 1: Objectives of the project and status

- 11. Clear statement of objectives that have not been achieved so far but will be achieved during the balance period: NA
- 12. Deviation from the approved objectives and reason thereof: NA
- 13. Whether mid-term progress report, final project completion report, financial papers (Utilization Certificate and Statement of Expenditure) submitted to funding agency? If not, reason thereof: Yes

14. Outcome of the project:

- Publications: Nil
- Patents: Nil
- Manpower trained in the project: 01

15. Table 2: Financial implications:

		Items	Total	Total amount	Funds	Any
			Sanctioned	released from funding	Utilized	deviations
			Amount	agency till date		
	20	Equipment	3.25 Lakhs	3.25 Lakhs	3.23	1,785
	ring				Lakhs	
-uoN	recur					
		Manpower	5.40 Lakh	5.30 Lakhs	5.3 Lakhs	9,619
s		Consumables	2.50 Lakhs	2.50 Lakhs	2.46	3,698
Head					Lakh	
rring		Travel	1.50 Lakh	1.50 Lakhs	99,983	50,017
Recu		Contingency	2.50 Lakhs	2.50 Lakhs	166,897	83,103

Introduction

Indian paper industry ranks 15'^ globally in respect of size, contributing more than 15 million tons of paper per annum. The industry accounts only 4 % of the global paper production of 404 million tons annually. The paper industry in India is considered to be highly polluting and categorized under the 17th most polluting industries, consuming large quantities of water as high as up to 80-100 cubic meters per ton of paper and discharge large volumes of effluents which are complex in nature containing dissolved organic as well as inorganic pollutants. The major environmental issues related to the Indian paper industry includes high consumption of fresh water and larger volumes of effluent discharge having high pollution loads in the form of color, COD and AOX (absorbable organic halides). The intensity of the pollution in the effluent discharges depends largely on the type of the raw material, manufacture process and chemicals used as well as process conditions and compounds formedduring pulping, bleaching and paper-making process. The pulp and paper millindustries generate wastewater that have very high biological oxygen demand (BOD), chemical oxygen demand (COD), toxic substances, recalcitrant organics, turbidity, high temperature and intense brown colour (due to the presence of lignin). Several physical and chemical processes for colour removal extensively studied till date include a combination of ultrafiltration, reverse osmosis techniques, ion exchange chromatography and lime precipitation, but all these processes are expensive and are not economical. Compared with physicochemical ways, biological methods for wastewater treatment are of cost benefit, ecofriendly and suitable for reduction of the BOD and COD from the effluents. However, the conventional biological processes have not effectively performed for removal of colour and recalcitrant compounds from paper and pulp wastewater. Though several works havebeen carried out using algae or bacteria or mixed culture of algae or bacteria for potential reduction of colour or other water pollutants from paper mill effluents, but these treatment processes are either very time consuming or only use to see the reduction of colour or AOX at lab scale. The success of these studies at pilot/industrial scale has not been reported and applied.Very limited work has been done on the removal of colour from the paper and pulp effluent. Generally, the industries discharge their effluent without proper treatment. Now, it has become mandate to remove colour of effluent before its discharge into the environment. Therefore, the aim of the present study is to develop economical and self-sustainable biological treatment technique in combination with fly ash nanoparticle which will be

efficient enough in the removal of effluent contaminants in terms of BOD, COD, AOX and colour from the effluent of paper mill.

Results

Objective 1: Characterisation of paper mill effluent

Using agro-residues as the feedstock, pulp and paper mill effluent was collected from a medium-sized mill in Uttarakhand (30.0668° N, 79.0193° E). Samples were taken in presterilized plastic containers consisting of fluorinated polymers like poly-tetrafluoroethylene (PTEF). The samples were taken from the secondary clarifier outlet discharge site (SC outlet). Composite sampling was carried out for 24 hours in order to obtain true and representative samples. To reduce the potential for volatilization or biodegradation between sampling and analysis, samples were collected in clean containers and transferred to the laboratory within 6 hours under a 4°C refrigeration environment.

According to conventional methods of analysis, the effluent was analysed for polluting characteristics such as BOD, COD, DO, TSS, TS, TDS, colour, total alkalinity, total acidity, and chloride analysis.

S.No.	Parameters	Effluent from	Effluent from	Permissible limit
		WWTP I	WWTP II	(for inland surface
		(Collects from	(Collects from	water) [given by
		entire city)	restricted area)	WHO]
1	рН	7.80	7.53	6.5-7.5
2	Temperature (°C)	38	38	35-37
3	Odour	Pungent	Pungent	Odourless
4	Colour	Grey	Light grey	Colourless
5	Electrical conductivity	2.30±0.02	2.24±0.05	0.6
	[EC] (mScm ⁻¹)			

Table 3: Water quality parameters of the collected effluent

6	Total dissolved solids [TDS] (mgL ⁻¹)	1160±1.8	1127±2.2	500
7	Total suspended solids [TSS] (mgL ⁻¹)	70.076±0.23	60.044±1.6	200
8	Total solids [TS] (mgL ⁻¹)	1240±1.5	1200±1.2	700
9	Total alkalinity [TA] (mgL ⁻¹)	32.833±0.04	32.824±0.1	40
10	Turbidity (NTU)	82.022±0.002	75.013±0.001	10
11	Total hardness [TH] (mgL ⁻¹)	4920±0.22	4120±0.14	600
12	Calcium hardness (mgL ⁻¹)	120±0.31	85±0.2	50
13	Dissolved oxygen [DO] (mgL ⁻¹)	2±0.08	3±0.2	4-6
14	Biochemical Oxygen Demand [BOD] (mgL ⁻¹)	180±1.8	122±1.5	30
15	Chemical Oxygen Demand [COD] (mgL ⁻¹)	390±1.5	345±1.1	250
16	Sulphate (mgL ⁻¹)	122±0.005	100±0.01	200
17	Phosphate (mgL ⁻¹)	2±0.02	1.8±0.01	10
18	Total nitrogen (mgL ⁻¹)	80±1.1	73±1.02	40

Objective 2: Screening and identification of lignolytic bacteria from <u>effluent</u>

Bacterial colonies were isolated by serial dilution, spreading and quadrant streaking. 1 mL aliquot of the stock solution (solution0) was added to tube 1 which contains 9 mL of autoclaved distilled water (dilent10⁻¹); the product of this mixture is solution1. Repeated by aliquoting 1 mL of the newly created solution1 and adding it to tube 2 which contains 9 mL of distilled water. This mixture has a dilution of 10⁻². Aliquoting and resuspension was continued in this manner until the final tube is reached, diluting the stock concentration by a factor of 10 each with each step. The final tube haddilution of 10⁻⁶. Each of these dilutions were spread on nutrient agar plates. The colonies that grow on the spread plate arise from a single cell and each colony on the dish can be counted to estimate the number of colony forming units per milliliter (CFU) in each suspension, represented as CFU/mL. Each of these colonies were then subsequently streaked in a quadrant on nutrient agar plated. Streaking was repeated until pure colonies were obtained, followed which glycerol stocks were prepared for long term storage.

A total of 93 bacterial colonies were isolated from the collected mill effluent and biochemical characterisation was performed for each one. The names of the biochemical tests were abbreviated as:

C.T.- Catalase test

- S.C.- Simmon's citrate agar test
- F.C.- Fermentation of carbohydrates (glucose)
- H₂S- Hydrogen sulfide detection test
- G.P.- Gelatinase production test
- A.T.- Amylase test
- M.R.- Methyl red test
- V.P.- VogeusPraskeur's test

Table 4: Summarized results of the biochemical tests on microbial isolates

Bacteria/	C.T.	S.C.	F.C.	H ₂ S	G.P.	A.T.	M.R.	V.P.
Test								
CP 1	+	-	+	-	+	-	+	-
CP 3	+	+	+	-	+	+	-	-
CP 4	+	+	-	-	+	+	+	-
CP 5	+	-	-	-	-	+	+	+
CP 6	+	+	-	-	+	+	-	+
CP 7	+	+	+	-	+	+	+	-
CP 8	-	+	-	-	-	+	+	-
CP 9	+	+	-	-	+	-	-	+
CP 10	+	-	-	-	-	-	-	+
CP 11	+	+	+	-	+	-	+	-
CP 12	+	+	+	-	+	+	+	-
CP 13	+	-	+	-	+	-	-	-
CP 14	+	+	+	-	+	-	-	+
CP 15	+	-	-	-	+	-	-	+
CP 16	+	-	-	-	+	+	-	-
CP 17	-	+	-	-	+	-	+	-
CP 18	-	+	-	-	+	-	+	-
CP 19	-	-	+	-	+	-	+	-
CP 20	+	+	+	-	+	+	-	+
CP 21	+	+	+	-	+	-	+	-

CP 22	+	+	-	-	+	-	+	-
CP 23	+	+	-	-	+	+	-	-
CP 24	+	-	-	-	+	-	-	+
CP 25	+	+	-	-	+	-	-	-
CP 26	+	+	-	-	+	-	-	+
CP 27	+	-	-	-	+	-	+	+
CP 28	+	+	-	-	+	-	+	-
CP 29	+	-	-	-	+	-	-	+
CP 30	+	-	+	-	+	+	-	+
CP 31	-	+	+	-	+	+	+	-
CP 32	-	+	+	-	+	+	+	-
CP 33	-	-	+	-	+	-	-	+
CP 34	+	+	+	-	+	+	+	-
CP 35	+	+	+	-	+	-	+	-
CP 36	+	+	+	-	+	-	-	-
CP 37	+	+	-	-	+	+	+	-
CP 38	+	-	-	-	+	-	-	+
CP 39	+	+	-	-	+	-	-	+
CP 40	+	+	+	-	+	-	+	-
CP 41	+	-	+	-	+	+	-	-
CP 42	+	+	+	-	+	-	-	+
CP 43	+	-	+	-	+	+	+	-

CP 44	-	-	-	-	+	-	+	-
CP 45	+	-	+	-	+	-	-	-
CP 46	+	+	+	-	+	-	+	-
CP 47	+	-	+	-	+	-	+	-
CP 48	+	+	-	-	-	-	-	+
РР	+	+	+	-	-	-	-	+
PP 1	+	+	+	-	-	-	-	+
PP 2	-	-	+	-	+	-	+	-
PP 3	-	+	-	-	-	-	-	-
PP 4	+	-	-	-	+	-	+	-
PP 5	+	+	+	-	+	-	-	+
PP 6	+	+	+	-	+	-	-	+
PP 7	+	+	+	-	+	-	-	+
PP 6	-	+	+	-	-	+	-	+
PP 7	+	+	+	-	+	-	-	+
PP 8	+	-	+	-	+	-	-	+
PP 9	+	+	-	-	+	+	+	-
PP 10	+	-	+	-	+	-	-	+
PP 11	+	+	_	-	+	+	+	-
PP 12	+	+	+	-	-	-	+	-
PP 13	+	+	+	-	+	-	-	+
PP 14	+	+	-	-	-	-	-	+
	1	1	1	1	1		1	

PP 15	+	+	+	-	-	-	-	+
PP 16	-	-	+	-	+	-	+	-
PP 17	-	+	-	-	-	-	-	-
PP 18	+	-	-	-	+	-	+	-
PP 19	+	+	+	-	+	+	-	+
PP 20	+	+	+	-	+	-	-	+
PP 21	+	+	+	-	+	-	-	+
PP 22	-	+	+	-	-	+	-	+
PP 23	+	+	+	-	+	-	-	+
PP 24	+	-	+	-	+	-	-	+
PP 25	+	+	-	-	+	+	+	-
PP 26	+	-	+	-	+	-	-	+
PP 27	+	+	-	-	+	-	-	+
PP 28	+	+	-	+	+	-	+	-
PP 29	+	+	-	-	+	-	-	+
PP 30	+	+	-	-	-	-	-	+
PP 31	+	+	+	-	+	_	_	+
PP 32	+	+	-	-	+	+	+	-
PP 33	+	+	-	-	-	+	+	-
PP 34	+	+	-	-	+	-	-	+
PP 35	+	+	+	-	+	+	-	+
PP 36	+	+	-	-	+	-	-	+

PP 37	+	+	-	-	-	-	-	+
PP 38	+	+	+	-	+	+	+	-
PP 39	+	-	+	-	+	-	-	+
PP 40	+	-	-	-	+	-	+	-
PP 41	+	+	-	-	+	+	+	-
PP 42	+	+	+	-	+	-	-	+
PP 43	+	-	+	-	+	+	-	+
PP 44	+	-	+	-	+	+	+	-
PP 45	+	+	-	-	+	-	-	+

+ : Activity was observed. - : No activity was observed



Fig. 1: Biochemical tests of microbial isolates

Screening of bacteria for lignolytic activity

All the isolates were screened on lignin agar for zone of inhibition indicating lignin degradation activity. Laccase, LiP, and MnP activities were tested on the chosen bacterial isolates. For the purposes of observing the activity of laccase, LiP, and MnP, guaiacol, 0.05%, Azure-B, and phenol red were added to MSM agar plate. One loopful of an overnight bacterial culture was used as an inoculant, and the plate was then incubated for 5 days at 32°C. The isolate's LiP and MnP activity was determined by the decolorization of phenol red and Azure-B, respectively. On the guaiacol-MSM agar plate, the bacterial colony was brown, indicating laccase activity. Only one of the 93 isolates, PP50, could produce both LiP and MnP activity; as a result, PB50 was selected for the preceding investigation.

The 250 mL shake flasks used for the liquid culture studies contained 50 mL of culture medium. In pre-culture studies, the carbon source provided was either 10 g/L glucose, 5 mM guaiacol, 3 mM vanillin, and 4-HBA (4-hydroxybenzoic acid), 1 g/L DL supplemented with 5 g/L glucose, or 1 g/L lignin or DL (low-cell density experiments).For the DL (or unprocessed lignin) tests, a single colony of the relevant microorganism from 1 g/L DL plates was used to inoculate the flasks. Model compounds were used as the carbon source in shake flasks, and a predetermined amount of biomass was added after a pre-culture of 10 g/L glucose to provide an initial OD of between 0.2 and 0.5.The majority of the trials were run in sets of two. The flasks were stirred and incubated at 27 °C (180 rpm). For the purpose of monitoring the biomass density (OD-optical density), samples were taken at regular intervals at 600 nm. Supernatant obtained after centrifuging the lignin media culture was taken as blank. Out of the screened 93 isolates, only 7 bacteria showed weakly positive lignin degradation capacity.



Fig. 2: Lignin degradation test on kraft lignin plates

Microbial Consortia

The seven bacteria showing some ligninolytic activity were further screened for improvement

Sl.	Parameter	Before		Ba		Consortia		
No		Treatment	PP9	PP14	PP38	PP50	PP6	
1.	BOD	89	28	32	25	38	40	20
2.	COD	480	184	149	234	250	267	200
3.	DO	6.5	4	4.5	3	3.5	5	4
4.	Chlorides	754	700	665	550	600	680	480
5.	pH	8.2	8.2	8	7.9	7.85	7.7	7.8
6.	Color	860	600	550	280	340	410	250

in the water quality parameters.

Table 5: Water quality parameters after treatment with microbial consortia and individual bacteria

Objective 3: Collection and preparation of fly ash nanoparticles

In the treatment of paper and pulp mill effluent, coal fly ash (CFA) and nano-sized coal fly ash were used. CFA was obtained from an NTPC coal-based thermal power plant in Dadri, Gautam Budh Nagar, Uttar Pradesh (28035'54"N 77036'34"E) and used without any pretreatment. As adsorbents for wastewater treatment, three grades of coal fly ash were used: Electrostatic precipitator (ESP) fly ash (CFA-1), Nano fly ash (CFA-N) and CFA-2. CFA-2 was a mixture of ESP ash and bottom ash (coarse ash) that is unutilized and stored together in the vicinity of the power plant. ESP is the finest fly ash collected from the ESP chamber of the thermal power plant, whereas CFA-1 is a mixture of ESP ash and bottom ash (coarse ash) that is unutilized and stored together in the vicinity of the power plant.

At the National Metallurgical Laboratory (NML), Jamshedpur, nano-sized ash of coal flyash was produced by attrition processing of ESP coal fly ash. For fine (wet) grinding and mechanical activation of fly ash, a continuous type Attrition mill (Labstar, Netzch, Germany) was utilised. Mastersizer 3000, which uses the laser diffraction technique to detect particle size distributions from 10 nm to 3.5 mm utilising a single optical measurement route, was used to calculate the size of the adsorbents.

Objective 4: Optimisation of the experimental parameters for synergistic effects of bacteria as well as fly ash nanoparticles together for colour removal and other pollutants



Fig. 3: Effect of adsorbent dose of different fly ash on the effluent colour removal

To determine the adequate dose of the adsorbent, different quantities of a variety of fly ash were contacted with the paper and pulp effluent in respect to its colour removal for 60 min at room temperature and 80 rpm. The results are presented in Figure 1 and indicate that the effluent decolourization increases with increase in adsorbent dose.

The intensity of effluent's colour drastically reduced with increasing adsorbent concentration upto 100 g/L, however, the minimum colour intensity of 15 % was observed at 60 g/L. The colour removal effectiveness is pronounced in the adsorbent dose range of 50 g/L to 80 g/L after which it became constant due to saturation. The % of colour removal increases the maximum between 50 g/L to 60 g/L due to increase in the availability of absorption sites, but decreases afterwards, due to attainment of equilibrium, at 80 g/L. Results indicate that about 92.21% removal of colour was achieved using 60 g/L of nano coal fly ash while 91.9%, 85.1% removal was achieved using coal fly ash, and mound ash, respectively. Results came better in case of the nano sized coal fly ash as compared to the coarser one, probably due to larger surface area, thus more interaction and better efficiency.



Fig. 4: Effect of contact time on the effluent colour removal

Figure shows the influence of adsorption/contact time on effluent colour removal by various adsorbents. During the first 10-50 minutes, there was a quick decolorization, but there was no significant change in the rate of colour removal after that. The ability of an adsorbent to achieve equilibrium in a short period of time indicates its efficacy in wastewater treatment. At 60 minutes of interaction time, the observed colour reduction efficiency was 92.21 % for coal fly ash (nano size), 91.1 % for coal fly ash, 85.1 % for mound ash. When the contact time is increased to 70 minutes, the efficiency of coal fly ash (nano size) increases to 91 %, 89.7% for coal fly ash, 84.2 % for mound ash. Because the difference was minor, the contact period was set at 60 minutes for the current investigation.



Fig. 5: Effect of oscillation time on the effluent colour removal

Sl.	Parameter	Unit	Before	Coal Ash			CPCB Norm
No			Treatment	ESP	FSP	Pond	-
				Ash	Ash	Ash	
				11011	Nano		
					sized		
1.	BOD	Ppm	86	26	22	32	30
2.	COD	Ppm	596	167	148	205	250
3.	TSS	Ppm	197	97	85	105	100
4.	TDS	Ppm	1236	1260	1277	1245	2100
5.	DO	Ppm	6	6	5.9	6.1	-
6.	Chlorides	Ppm	845	845	847	848	1000
7.	рН		8.2	8.2	8.2	8.2	6.5-8.0
8.	Temp.	°C	28	28	28	28	Not exceeding 5 °C above the receiving water
9.	Color	PCU	878	165	82	113	100approx.(Nospecificstandard)

Table 6: Effect of Coal ash on different water quality parameters

The interaction of 50 g of various fly ash (adsorbents) with 1L of the paper and pulp effluent at different rpms ranging from rest (0 rpm) to 10-100 rpm was used to investigate the

influence of static and shaking circumstances on the reduction of effluent colour. Figure 3 shows that when the oscillation intensity increases from 10-100 rpm, the effluent colour decreases. However, only a modest colour shift in terms of decolorization was noticed at a static state or 0 rpm (result not shown in Figure 3). The colour removal efficiency rose as the oscillations increased, peaking at 92.2 % for coal fly ash (nano), 91.1 % for coal fly ash, 85.1 % for mound ash. Decolorization dropped to 91.4 % in coal fly ash (nano), 87.4 % in coal fly ash and, 83.6 % in mound ash.





Fig. 6: Experimental set ups of effluent treatment by different fly ash

Objective 5: Fabrication and optimization of the treatment system for <u>paper effluent at benchscale</u>



Fig. 7: Schematic representation of bioreactor for colour removal of pulp and paper effluent



Fig. 8: Pilot plant reactor fabricated for colour removal of pulp and paper effluent

The system consists of Tank - I, which has a capacity of approximately 5 litres, and the filtered effluent is transferred to Tank - II, which has a capacity of approximately 20 litres, is made of stainless steel transparent acrylic, has a 40 RPM agitator, and a secondary filter, as well as Pump - I to remove fly-ash. The liquid is moved to tank III, which is composed of stainless steel and has a cooling/heating system as well as a 40 RPM agitator (For retention, add enzymes and other ingredients). The entire system is under control. After the cycle is completed, the liquid is delivered to a tank - IV with a filter to separate the residuals using the control panel. As illustrated in the layout, all tanks and the control panel are installed on the pipe structure.

The secondary effluent collected from a paper and pulp industry was run through the pilot reactor at the determined optimal conditions from the batch experiments, i.e.,

Tank I- Fly ash treatment: 80 RPM, 25°C, 60 g/L fly ash dose, 60 min

Tank II- 0.8 OD of overnight grown culture of the microbial consortia, 12 hours.

After a treatment cycle, the brown colour of the effluent almost disappeared, and the effluent appeared transparent.



Fig. 9: Image of colour removal of the effluent post treatment with fly ash only and full treatment

Objective 6: Assessment of toxicity of the treated water by cytogenotoxicity

A simple and cost-effective approach of assessing germination and growth parameters of Vigna radiata seeds was used to examine effluent treated with coal fly ash (in comparison to negative and positive controls). The growth characteristics and percent germination (percent) were assessed after 120 hours (5 days) of incubation of 10 seeds each treated with distilled water, raw, and 25 treated effluent, as shown in Table 7. The effect of distilled water on seed germination was 100% (Fig 10a), but following exposure to raw effluent, the effect was severely reduced to 40% (Fig 10c). The length of the plumule and radicle was similarly lowered to 3.30.18 cm and 0.40.11 cm, respectively (which was quite less from that observed in case of distilled water). The effluent's toxicity decreased significantly after treatment with fly ash, as evidenced by an increase in seed germination to 70%, as well as an increase in plumule growth to 5.610.14 cm and radicle growth to 1.90.06 cm, possibly due to degradation of the toxic organic pollutants and xenobiotics present in the raw effluent.

Parameters	Seeds exposed to distilled water (Negative Control)	Seeds exposed to raw effluent (Positive Control)	Seeds exposed to treated effluent
Plumule length (cm)	7.45 ±0.30	3.3±0.18***	5.61±0.14***
Radicle length (cm)	2.25 ±0.09	0.4±0.11***	1.9±0.06***
Germination (%)	100	40***	70***

Table 7: Phytotoxicity assay on the seeds of Vigna radiata



Fig. 10: Vigna radiata seeds exposed to (a) distilled water, (b) raw effluent and (c) treated effluent

Genotoxicity of the treated effluent

The comet assay test reveals DNA damage, such as single or double stranded breaks, as a comet or tail attached to the nucleus of the cells migrating towards the anode. Due to significant toxicity, the appearance of a comet was seen in fluoresced images of root tip cells exposed to raw effluent (Fig 11b), as opposed to non-toxic distilled water treated root tip cells. After treatment of effluent with coal fly ash (Fig 11c), the extent of comet length as well as other comet parameters (Table 8) were reduced to a reasonable extent, demonstrating the effectiveness of the laboratory prepared ash in reducing the toxicity of paper and pulp wastewater and allowing its reuse.

Table 8: Detection of the extent of DNA damage in the nuclei of *Allium cepa* root tip cells using Comet assay

Parameters	Onions exposed to	Onions exposed to raw	Onions exposed to
	distilled water	effluent	treated effluent
	(Negative Control)	(Positive Control)	
Tail DNA (%T)	16.710±1.11	98.23±1.12***	42.48±1.72***
Tail length (µm)	15±1.25	90±1.42***	49±1.88***
Tail moment	2.44±1.21	78.22±1.28***	44.5±1.13***
Olive tail moment	0.97±1.08	40.31±2.34***	25.9±2.12***



Fig. 11: Comet characters observed in root tip cells of Allium cepa grown in (a) distilled water, (b) raw effluent and (c) treated effluent

Outcomes

- 1. Batch studies successfully showed colour removal upto 91%, BOD, COD reduction by coal fly ash as well as microbial consortia.
- 2. A pilot-scale bioreactor was fabricated.
- 3. Much colour reduction from pulp and paper effluent achieved after treatment in the pilot scale reactor.

Number of Ph.D. produced- 1: Kavya Bisaria

Publications:

"A sustainable approach for treatment of Agro-based mill effluent and waste management" (Communicated)

Conference:

Keynote lecture- "A synergistic and economical approach for treatment of pulp and paper mill effluent system using microbes and fly-ash nanoparticles to achieve minimum/zero waste discharge" by **Prof. Dr. Rachana Singh**at the international conference on "Promoting Environmental Technologies for Waste Management and Sustainable Development (WMSD-2021)

Oral Talk- "Magnetic biochar and indigenous bacteria immobilized alginate beads for paper and pulp mill effluent treatment" at the international conference on "Promoting Environmental Technologies forWMSD-2021", Bhubaneshwar, Orissa- Kavya Bisaria, Ashwani Dixit and Rachana Singh



Image: Pilot plant reactor- (L to R: Ms. Kavya Bisaria, Dr. Rachana Singh (PI- Amity University), Ms. Shruti, Mr. Kumar Anupam (Scientist B- CPPRI)).

Media Coverage: Report published in "Kargad Bharti" (Image below)

