How to Cite:
Garg, A. K., Faheem, M., & Singh, S. (2022). Optimization of process variables for reduction of genotoxicity from paper mill effluent. *International Journal of Health Sciences*, *6*(S3), 1995–2007. https://doi.org/10.53730/ijhs.v6nS3.5915

Optimization of process variables for reduction of genotoxicity from paper mill effluent

Amit Kumar Garg
Department of Biotechnology, School of Life Sciences, Singhania University, Pacheribari, Jhunjhunu, Rajasthan, India

Mohammed Faheem
Department of Biotechnology, School of Life Sciences, Singhania University, Pacheribari, Jhunjhunu, Rajasthan, India

Sumer Singh
Department of Biotechnology, School of Life Sciences, Singhania University, Pacheribari, Jhunjhunu, Rajasthan, India

***Abstract*---**In recent years, microbial techniques for wastewater degradation process optimization under laboratory circumstances have piqued the interest of many academics acress the word, prompting them to investigate practical, cost- effective and long-term wastewater treatment technologies. Some workers claim that certain metabolic compounds have mutagenic nature and endocrine disrupting properties. To confirm the degradation and identification of a variety of metabolic products that may have EDC properties, control and bacterial treated samples were analyzed with the help of HPLC and GC-MS.In this study, toxicity assessment (genotoxicity) were performed to confirm the load of various EDCs present in the RGP effluent.Glucose was the most abundant among the carbon sources used as it showed the greatest reduction in colour (61.93%) and COD (57.86%) at 96 hours of the incubation period. However, galactose and sucrose were also found to be very good, allowing 56% color and 48.4% COD reduction. Sodium nitrate, which is an inorganic nitrogen source, played an important role in pulp paper mill effluent decolorization as well as COD reduction. On the other hand, a negative contribution to the color reduction was shown by the inorganic nitrogen source ammonium nitrate. The untreated paper mill effluent shows higher toxicity which expressed in terms of olive tail moment (OTM) and percent (%) tail DNA (6.13±0.27 and 20.31±1.08). This genotoxic nature of untreated paper mill effluent is may be due to high load of EDCs which are present in untreated paper effluent. Finally this study concluded that the isolated bacteria

International Journal of Health Sciences ISSN 2550-6978 E-ISSN 2550-696X © 2022.
***Corresponding author**: Faheem, M.; Email: faheem.biochem@gmail.com*
Manuscript submitted: 18 Nov 2021, Manuscript revised: 09 Feb 2022, Accepted for publication: 27 March 2022

have potential to remove different toxic pollutants in mixed condition rather than single culture. The developed bacterial strains were identified as *Serratia marcescens* and *Bacillus megaterium* on the basis of primary and secondary biochemical test. These two bacteria were subjected into optimization of process parameters. *Serratia marcescens* and *Bacillus megaterium*capable for more than 80% reduction of color of pulp paper mill effluent at optimized conditions.

***Keywords*---**wastewater, degradation process, optimization, toxicity, genotoxic.

Introduction

During the paper making process, the pulp and paper mills consume a lot of water and several chemicals. It also generates a large amount of wastewater, which contains high levels of suspended solids, tannins, resin acids, heavy metals(magnesium, sodium, chlorides), sulphur compounds and lignins as well as high levels of BOD, COD and potentially toxic chlorinated compounds, suspended solid tannins, resin acids, heavy metals(magnesium, sodium, chlorides) and sulphur compounds (Haq et al. 2016). In India, there are 759 pulp and paper factories with many them employing chlorine compounds in their bleaching processes. The Indian paper and pulp busness use a lot of water, using freshwater per tonne of paper and generating between 75 and 225 m^3 of effluent per tonne of paper (Tewari et al., 2009). Sodium hydroxide, sodium carbonate and chlorine compounds are used by pulp and paper mills during the washing, pulping and bleaching processes (Kumar et al., 2020b).The primary pollutants created by the paper industries are chlorinated phenils, lignin and their derivatives (Raj et al, 2007). Lignin is a key recalcitrant component that causes off- putting colour and inhibits phototrophic speciies developments by decreasing sunlight transmission in waste water (Karrasch et al 2006). Furthermore, chlorinated chemicals and organic halides can bioaccumulate in the tissues of fish causing carcinogenic, clastogenic, endocrine and mutagenic endocrine consequences (Kumar et al 2020b). Through the food chain, people may be exposed to these contsminated fish, posing a number of major health risks (Savants et al., 2006).

In recent years, microbial techniques for wastewater degradation process optimization under laboratory circumstances have piqued the interest of many academics acress the word, prompting them to investigate practical, cost- effective and long-term wastewater treatment technologies (kaushik et al., 2020). Most fungi have significant ligninolytic activity due to the production of microbial enzymes such as laccase, lignin peroxidise and manganese peroxidise(Kumar and Chandra, 2020). However, due to the low pH range (3-5). Lengthy development period, high spore generation and unfavourable dubmerged circumstances for fungal growth, wide- scale applications of these approaches are limited (Arimi et al ., 2014). The pH value of pulp paper mill wastewater (PPMW) are usually alkaline (pH 7-9), and the necessity to lower the pH before fungal inoculation adds to the cost. Bacterial that can survive in an acidic to alkaline pH range, unlike fungi, can play an important role in the bioremediation of PPMW without

requiring pH adjustment. Bacterial laccases may use a wide variety of substractes and are active at high pH and temperatures (Kumar and Chandra, 2020). Several prior researchers discovered bacterial species capable of degrading lignin, PPMW and detoxifying (Chandra et al., 2007). However, there remainded a lnowlwdge gap concerning ligninilytic anzymes capable of degrading and detoxifying PPMW under optimal environmental and nutritional circumstances (Wang et al., 2010).

We know that any technology needs to be optimized before it can be implemented. The optimization is done in such a way that we get maximum benefit from the work done. In addition, there is little information about the influencing factors for in-situ biodegradation application of persistent organic pollutants in nature that may enhance this degradation process. This is why we have focused in this section on important influencing factors such as pH, RPM, inoculation volume of bacterial suspension, carbon source (glucose, dextrose, lactose, galatose), nitrogen source (peptone, beef extract, yeast extract). These important influencing factors can increase or decrease the growth of bacteria. In recent years, scientists have characterized a range of metabolic products of paper effluent (Chandra and Singh, 2012; Haq et al., 2017). Some workers claim that certain metabolic compounds have mutagenic nature andendocrine disrupting properties. To confirm the degradation and identification of a variety of metabolic products that may have EDC properties, control and bacterial treated samples were analyzed with the help of HPLC and GC-MS.In this this study, toxicity assessment (genotoxicity) were performed to confirm the load of various EDCs present in the RGP effluent.

Materials and Methods

Description of sample and sampling methods

The rayon grade pulp paper mill effluent samples were collected from main outlet channel of *M/S* Century Paper mill, lalkuan, Nainital, Uttarakhand, India in pre-sterilized container (10 litter capacities). Samples were brought to the laboratory and keep under 4°C until analysis. This RGP effluent sample was used for physic-chemical analysis, biodegradation studies and characterization of organic pollution before and after degradation experiment.For isolation of aerobic bacteria, the sludge sample was collected in a 500 mL beaker at the same location.

Chemical texture of pulp paper mill effluent

The physic-chemical analysis such as total solid, dissolve solid of RGP effluent was done according to the standard protocol given in APHA, 2005 (American Public Health Association).The pH and electrical conductivity (EC) of effluent sample was analysed with the help of selective electrode (Thermo Orion Model 960). Before EC measurement the electrode was calibrated at room temperature (25°C) with the standard (1413 µS/cm and 12900µS/cm). The alkalinity of sample was done by titration method (Method number: 2320B, APHA, 1998) with the help of standard solution of sulphuric acid solution (0.1N). Other parameters such as color by Cobalt Platinum method, Phosphate by Stannous chloride method,

Sulphate by Turbidimetric method, Biological oxygen demand (BOD) by 5-day test and Chemical oxygen demand (COD) by Open reflex method were analyzed.

Media composition and source of bacteria

The Final effluent amended with 0.5% glucose and 0.1% peptone having following characteristics: lignin: 2283 mg/l, Color: 1995 copt, BOD: 1288 mg/l, COD: 3092 mg/l, sulphate: 750 mg/l, nitrate: 20.77, iron: 9.08, nickel: 2.01, lead: 1.01 and Phosphate: 35 was used for final screening and further bacterial degradation study.

Isolation, screening and identification of potential bacteria

One gram sludge sample was transfer aseptically to final effluent. Flasks were kept on shaking incubator at 120 rpm for ten days at 30°C (New Brunswick, Innova-4230 USA). When sample showed reduction in color and COD then we took 0.1 ml sample and spread on lignin amended solidified plates with 15 gm/L agar (HiMedia, India). Plates were kept in incubator at 36 °C until colonies developed. Twelve purified bacterial strains designated as XPB1, XPB2, XPB3, XPB4, XPB5, XPB6, XPB7, XPB8, XPB9, XPB10, XPB11 and XPB12 were screened first on the basis of presence and absence of carbon and nitrogen source and secondon the basis of their COD, color and lignin reduction potential. Further, identification of final screened bacteria was further subjected into identification process by classical biochemical methods. Two bacteria designated as XPB4 and XPB6 showed highest ability to remove pollution parameters and were further identified by biochemical method as *S. marcescens*, and *Bacillus megaterium*, respectively. These selected bacteria were used for further bacterial treatment studies.

Removal of toxic pollutants by axenic and co-culture condition

Transfer a 4% (v/v) overnight grown bacterial suspension of constructed co-culture (inoculum size 2.9 x 105 and 2.6 x 105 for *S. marcescens*, and *Bacillus megaterium*, respectively) aseptically to the 250 ml flask containing a 95 ml RGP final effluent. Samples were taken every day and end of experiment and analyzed for pollution parameters (colour, lignin, COD and BOD) and toxicity assessment. During the treatment of RGP effluent, the dynamics of cell growth (by cfu/ml), pH and enzyme activity (Chandra et al., 2011) of bacterial strains were analysed. The BOD/COD ratio was also calculated by method given by Kreetachat et al. (2007).

Metabolite characterization by HPLC and GC-MS

After bacterial treatment, degraded samples with their respective control were centrifuged at 10,000 rpm and acidified (pH 1-2) with 0.1 HCl. for qualitative estimation of organic compounds from RGP effluent was done according to method of Abhisek et al., 2017 with minor modification. In brief, 50 ml of was taken degraded samples with their respective control in separating funnel (100 ml) extracted with 50 ml ethyle acetate by shaking it at room temperature (35 °C) for 15 minutes. Organic layer was pooled together, collected and dried by using vacuum evaporator. Sample was reconstituted to 1ml prior to injection and ready

for further studies (HPLC and GC-MS). For HPLC samples were analyzed using a (Waters, 515 HPLC), equipped with 2487 UV/VIS detector, via millennium software. A 10 µl Sample was njected followed by implementation of HPLC grade acetonitrile/ water (70:30) at a rate of 1 ml/min. Reverse phase C-18 column at 27 °C were used to analyze lignin at 280 nm. For GC-MS analysis dry residues of ethyl acetate extracts were analyzed as trimethylsilyl (TMS) as described (Lundquist and Kirk 1971). The identification of low molecular weight lignin-related compounds derived after bacterial treatment was done by comparing their mass spectra with that of the NIST library available in the instrument and by comparing the retention time (RT) with those of authentic compounds available.

Genotoxicity evaluation after bacterial treatment
Cell Line and their maintenance

The main goal of this study was to assess the induced toxicity of RGP effluent on human epidermal keratinocyte cell line. This cell line is mainly used for evaluation of toxic potential for different chemical compounds. The toxicity profile of RGP effluent is very important for the evaluation of induced degree of toxicity on human, because ultimately human will affected by this effluent in different way. Hence, in this study toxicity test was performed with this cell line (Abhishek et al., 2017).

Genotoxicity assessment

Cells were grown in 96-multiwell black plates (2 x 10^5 cells/well). This 96 well plate were treated with four types of samples (CM-control/DMSO alone; untreated rayon grade paper mill effluent: UT; bacterial treated sample after 5^{th} day: BT5 and bacterial treated sample after 9^{th} day BT9). After completion of treatment time, the exposed cells were mixed with low melting agarose and spread on slides. The slides were incubated in lysis solution for overnight at 4 °C. They were washed with electrophoresis buffer and further incubated in the same buffer for 20 minute. Then electrophoresis was performed for 25 min at 22 Volt. After electrophoresis the gels were neutralized in neutralization buffer. Finally, the slides were stained with EtBr (15 µg/ ml) for 5 min and dipped in chilled distilled water to wash off excess EtBr and cover slip placed in dark humidified box to prevent drying of the gel. Slides were scored using an image-analysis system (Kinetic Imaging, Liverpool, UK) attached to a fluorescence microscope (Leica, Germany) equipped with appropriate filters (N2.1 excitation wavelength of 515-560 nm and emission wavelength of 590 nm). The comet parameters recorded were olive tail moment (OTM, arbitrary unit) and tail DNA (percent %).

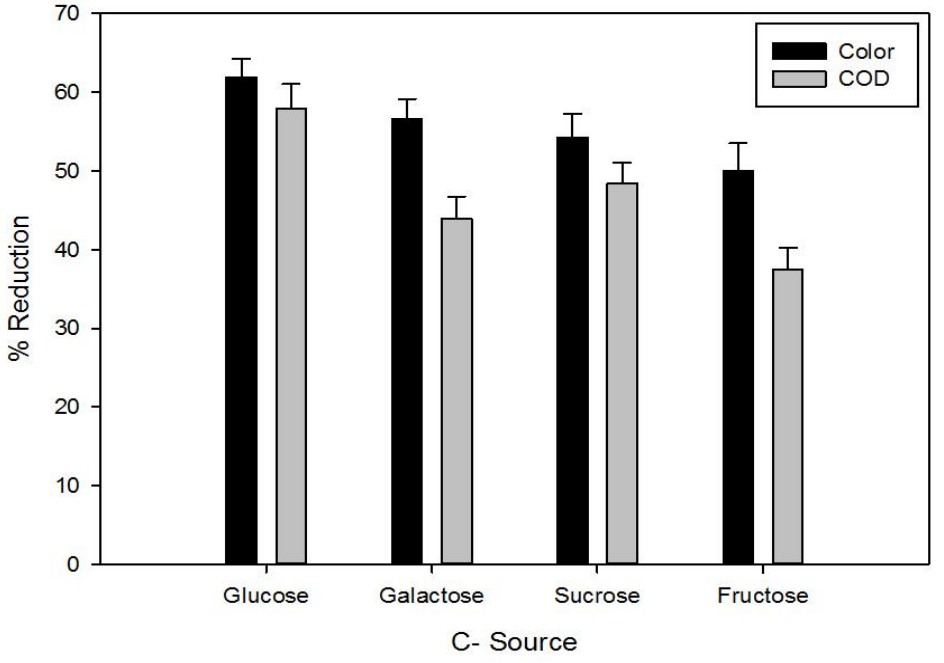
Statistical analysis

Numerical data were presented in the form of mean and standard deviation. One way ANOVA with post hoc test were applied to analyze the significant difference in among physico-chemical parameters in screening and genotoxicity. Statistical analysis was carried out using the statistical package for the social science, version 22 (SPSS-22, IBM, Chicago, USA). The level of significance that means, p value was set as <0.05. Graphics were prepared by the help of SPSS and Prism Software.

Results and Discussions

Optimization of process variation for reduction of pollution from pulp paper mill effluent with co-culture bacterial strains. We know that any technology needs to be optimized before it can be implemented. The optimization is done in such a way that I get maximum benefit from the work done. In addition, there is little information about the influencing factors for in-situ biodegradation application of persistent organic pollutants in nature that may enhance this degradation process (Gönder et al., 2012; Mishra et al., 2017; Mishra and Thakur, 2010; Pedroza-Rodríguez and Rodríguez-Vázquez, 2013). This is why we have focused in this section on important influencing factors such as pH, RPM, inoculation volume of bacterial suspension, carbon source (glucose, dextrose, lactose, galatose), nitrogen source (peptone, beef extract, yeast extract). These important influencing factors can increase or decrease the growth of bacteria. For the checking of reduction of pollution, two basic parameters namely color and COD were measured throughout the experiment.

Glucose was the most abundant among the carbon sources used as it showed the greatest reduction in colour (61.93%) and COD (57.86%) at 96 hours of the incubation period (Figure 4.8). However, galactose and sucrose were also found to be very good, allowing 56% color and 48.4% COD reduction. Of all the carbon sources, fructose was a relatively poor carbon source, causing 50% of the color and only 37% of COD reduction (Figure 1). Therefore, the order of the effective carbon sources was as glucose > galactose > sucrose > fructose. Likewise, the sequence of nitrogen sources tested was as peptone > beef extract > sodium



nitrate > ammonium nitrate

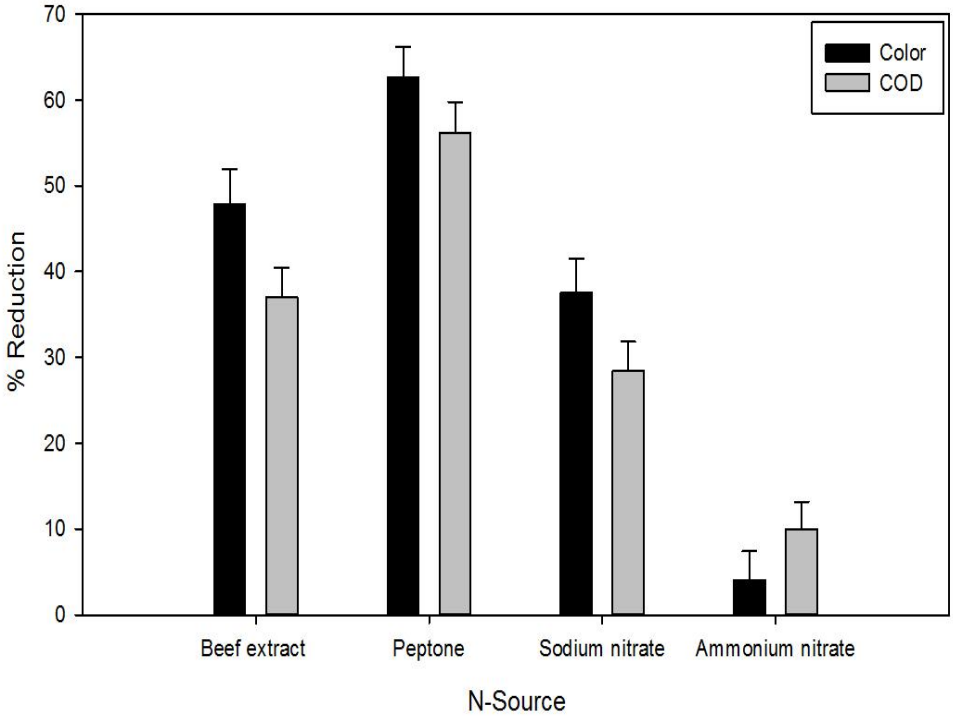


Figure 1. Optimization of carbon and nitrogen source for reduction of pollution parameters from pulp paper mill effluent

Sodium nitrate, which is an inorganic nitrogen source, played an important role in pulp paper mill effluent decolorization as well as COD reduction. On the other hand, a negative contribution to the color reduction was shown by the inorganic nitrogen source ammonium nitrate Figure 1. These results suggested that organic nitrogen sources favour the reduction of pollution parameters and bacterial growth over inorganic nitrogen sources. Similar to this study, the negative effect of inorganic nitrogen on wastewater treatment has also been reported by Xu et al. (2018). The reduction in the color of pulp paper effluent has also been reported by various authors, but very few studies have adapted to environmental and nutritional conditions (Morii et al., 1995; Jain et al., 1997; Gupta et al., 2001; Gomathi et al., 2012). Once the carbon and nitrogen sources were optimized, we switched to other process parameters. The inoculum size of the co-culture also affects the color reduction of the paper effluent. A maximum reduction in color (64%) and COD (56%) was noted at 4% of inoculum size and a further increase of 6% in inoculum size did not affect the color change process. After 4% of the size of the inoculum, the level of nutrients as well as oxygen in the bacterial culture medium decreases further. This reduction in culture media may affect the color change process as the size of the inoculum is further increased (Chandra et al., 2011) (Figure 2).

After optimization of the bacterial inoculum size, we optimize the agitation rate and the pH of the medium that most influences bacterial growth. In this study,

the maximum reduction in final effluent color (71 and 72%) and COD (63, 64%) was noted at 140 rpm and pH 8 (Figure 3). This indicates that the bacteria growing in the effluent prefer to live in alkaline conditions and have a high dissolved oxygen requirement. A higher reduction in alkaline pH may occur because higher pH favours the solubility of lignins and their derivatives .The ambient temperature was 37±1 °C which was noted for optimal reduction of pollution parameters of the effluent (85% color and 79% COD) (Figure 4). Finally, the developed bacterial strains (*Serratia marcescens* and *Bacillus megaterium*) in co-culture condition were able to reduce the color of pulp paper mill effluent by more than 80% under optimized conditions (ie glucose: 0.5%; peptone: 0.5%; inoculum size: 4 %; agitation rate 140 rpm, 37 °C temperature and pH 8.0).

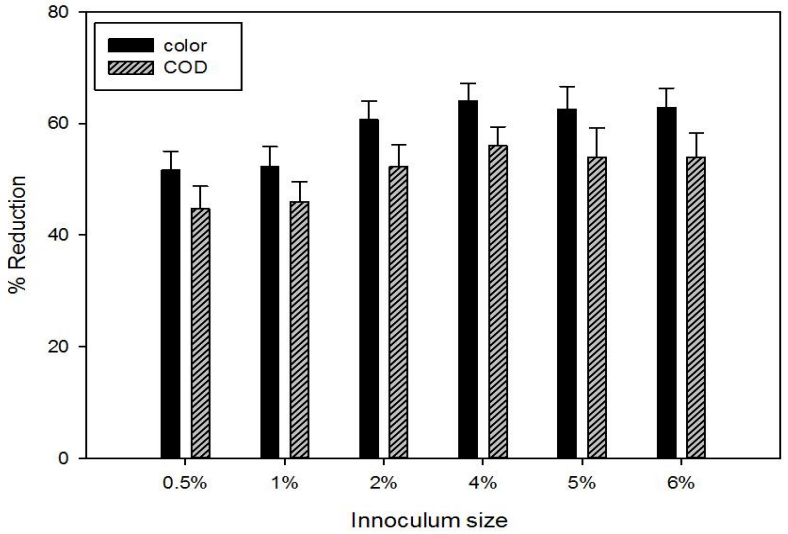


Figure 2. Optimization of bacterial load for effluent treatment

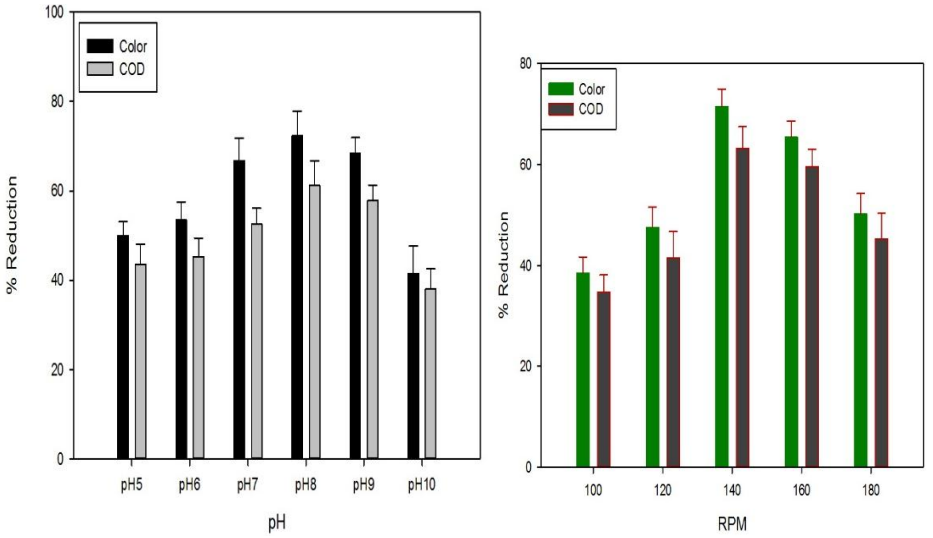


Figure 3. Optimization of agitation rate and pH for effluent treatment

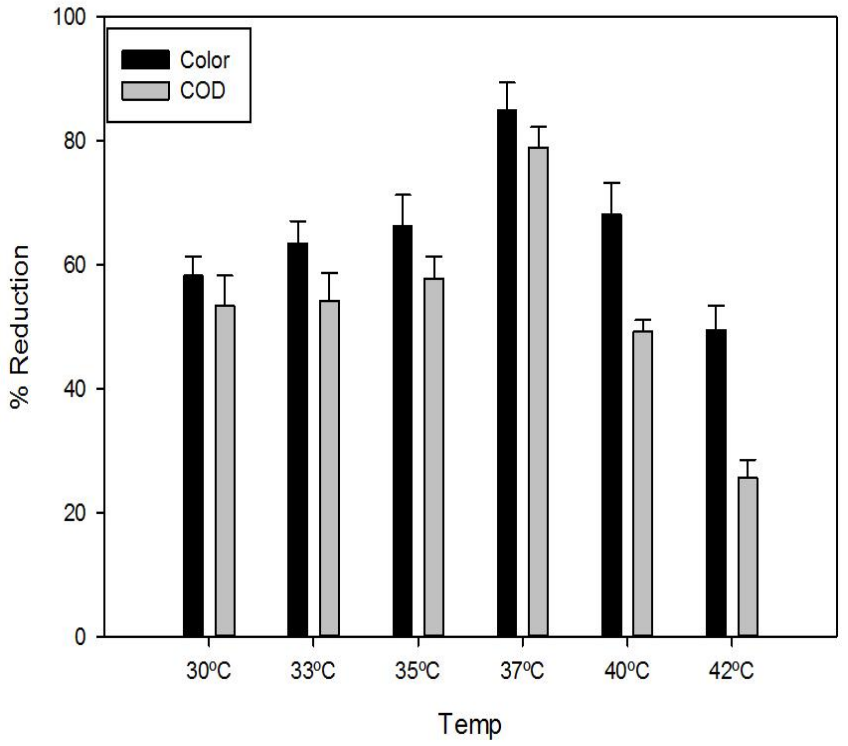


Figure 4. Optimization of Temperature for effluent treatment

Toxicity assessment (genotoxicity) for confirmation of reduction of different EDCs present in paper mill effluent

The comet assay, also known as single cell gel electrophoresis, is a simple but very sensitive technique for measuring brakeage of DNA stand. In this study, comet assay elucidated that DNA break down upon exposure to various chemicals extracted from untreated and bacterial treated pulp paper mill effluent. We have used medium control (Table 1). The medium control is used to observe the effect of process of single cell gel electrophoresis. The untreated paper mill effluent shows higher toxicity which expressed in terms of olive tail moment (OTM) and percent (%) tail DNA (6.13±0.27 and 20.31±1.08). This genotoxic nature of untreated paper mill effluent is may be due to high load of EDCs which are present in untreated paper effluent. For example chlorophenols, Phthalate and their derivatives which has high carcinogenic and mutagenic effect and may cause toxicity to living beings by inactivating respiratory enzymes, inhibiting oxidative phosphorylation and damage mitochondrial integrity (Rossi, et al., 2016). Moreover, high level of pentachlorophenol can cause blockage in the circulatory system of the lungs heart failure and even damage to the central nervous system. On other hand, heavy metals like Chromium, cadmium, arsenic also influence the toxicity at genetic level (Warren et al., 2081; Saha et al., 2011; DesMarias, T.L. and Costa, 2019). After bacterial treatment the OTM and percent

% tail DNA was reduces up to 55.4% and 43.87% (Figure 5). This reduction in genotoxicity indicated reduction of EDCs present in paper mill effluent.

Table 1
Genotoxicity of untreated and bacterial treated pulp paper mill effluent extract by alkaline comet assay

Samples	OTM (Arbitrary unit)	Tail DNA (%)
Medium control	0.28^{a}±0.04	6.04±0.11
Un treated paper mill effluent	6.13^{b}±0.27	20.31±1.08
Bacterial treated sample after 5^{th} days	4.08^{c} ±0.11	12.88±1.18
Bacterial treated sample after 9^{th} days	2.73^{d} ±0.7	11.4±0.82
Total reduction	55.4%	43.87%

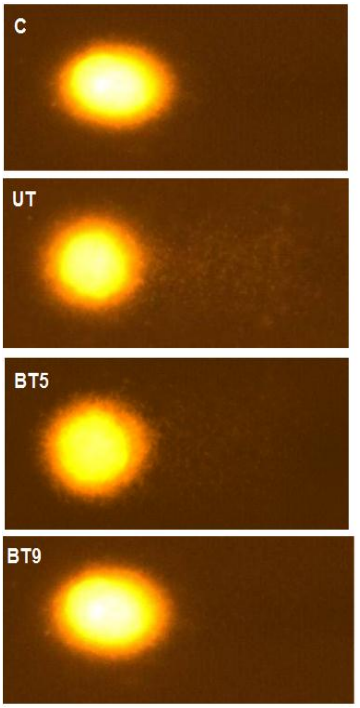


Figure 5. Comet assay of untreated (UT) and bacterial treated (BT-5 and BT-9: sample after 5^{th} and 9^{th} day) paper mill effluent samples; C: medium control.

Conclusion

Finally this study concluded that the isolated bacteria have potential to remove different toxic pollutants in mixed condition rather than single culture. The developed bacterial strains were identified as *Serratia marcescens* and *Bacillus megaterium* on the basis of primary and secondary biochemical test. These two

bacteria were subjected into optimization of process parameters. *Serratia marcescens* and *Bacillus megaterium* capable for more than 80% reduction of color of pulp paper mill effluent at optimized conditions (i.e. glucose: 0.5%; peptone: 0.5%; inoculum size: 4%; agitation rate 140 rpm, 37°C Temperature and pH 8.0). The HPLC and GC-MS analysis reveals that toxic pollutants removed by develop bacterial co-culture. Finally, the toxicity analysis of bacterial treated samples by the help of comet assay reveals that pollutants present in paper mill effluent was removed after bacterial treatment.

References

1. Abhishek, A., Dwivedi, A., Tandan, N. and Kumar, U., 2017. Comparative bacterial degradation and detoxification of model and kraft lignin from pulp paper wastewater and its metabolites. *Applied Water Science*, *7*(2), pp.757-767.
2. APHA, 2005. Standard Methods for the Examination of Water and Wastewater, twenty-second ed. American Public Health Association, Washington, DC.
3. Arimi, M.M., Zhang, Y., Gotz, G., Kiriamiti, K., Geißen, S.U., 2014. Antimicrobial colorants in molasses distillery wastewater and their removal technologies. Int Biodeterior Biodegrad 87, 34–43.
4. Chandra, R., Raj, A., Purohit, H.J., Kapley, A., 2007. Characterisation and optimisation of three potential aerobic bacterial strains for kraft lignin degradation from pulp paper waste. Chemosphere 67, 839–846.
5. Chandra, R., Abhishek, A. and Sankhwar, M., 2011. Bacterial decolorization and detoxification of black liquor from rayon grade pulp manufacturing paper industry and detection of their metabolic products. *Bioresource technology*, *102*(11), pp.6429-6436.
6. Chandra, R., Singh, R., 2012. Decolourisation and detoxification of rayon grade pulp paper mill effluent by mixed bacterial culture isolated from pulp paper mill effluent polluted site. Biochem Eng J 61, 49–58.
7. DesMarias, T.L. and Costa, M., 2019. Mechanisms of chromium-induced toxicity. *Current opinion in toxicology*, *14*, pp.1-7.
8. Gomathi, V., Ramanathan, B., Sivaramaiah, N., Ramanjaneya, V.R. and Jayasimha, D., 2012. Decolourization of paper mill effluent by immobilized cells of Phanerochaete chrysosporium. *Int J Plant Anim Environ Sci*, *2*(1), pp.141-146.
9. Gönder, Z.B., Arayici, S. and Barlas, H., 2012. Treatment of pulp and paper mill wastewater using utrafiltration process: Optimization of the fouling and rejections. *Industrial & engineering chemistry research*, *51*(17), pp.6184-6195.
10. Gupta, V.K., Minocha, A.K. and Jain, N., 2001. Batch and continuous studies on treatment of pulp mill wastewater by Aeromonas formicans. *Journal of Chemical Technology & Biotechnology: International Research in Process, Environmental & Clean Technology*, *76*(6), pp.547-552.
11. Haq, I., Kumar, S., Kumari, V., Singh, S.K., Raj, A., 2016. Evaluation of bioremediation potentiality of ligninolytic Serratia liquefaciens for detoxification of pulp and paper mill effluent. J. Hazard. Mater. 305, 190e199.

12. Haq, I., Kumar, S., Raj, A., Lohani, M. and Satyanarayana, G.N.V., 2017. Genotoxicityassessment of pulp and paper mill effluent before and after bacterial degradation using Allium cepa test. *Chemosphere*, *169*, pp.642-650.
13. Jain, N., Shrivastava, A.K. and Srivastava, S.K., 1997. Degradation of black liquor, a pulp mill effluent by bacterial strain Acinetobacter calcoaceticus. *Ind. J. Exp. Biol*, *35*, pp.139-143.
14. Kumar, V., Chandra, R., 2018. Characterisation of manganese peroxidase and laccase producing bacteria capable of degradation of sucrose glutamic acid-maillard reaction products at different nutritional and environmental conditions. World Journal of Microbiology and Biotechnology 34, 32.
15. Kumar, A., Chandra, R., 2020. Ligninolytic enzymes and its mechanisms for degradation of lignocellulosic waste in environment. Heliyon 6, e031702.
16. Kumar, A., Singh, A.K., Chandra, R., 2020b. Comparative analysis of residual organic pollutants from bleached and unbleached paper mill wastewater and their toxicity on Phaseolus aureus and Tubifex tubifex. Urban Water Journal 17, 860–870. https:// doi.org/10.1080/1573062X.2020.1836238.
17. Karrasch, B., Parra, O., Cid, H., Mehrens, M., Pacheco, P., Urrutia, R., Valdovinos, C., Zaror, C., 2006. Effect of pulp and paper mill effluents on the microplankton and microbial self-purification capabilities of the Biobio River Chile. Sci Total Environ 359, 194–208.
18. Kreetachat, T., Damrongsri, M., Punsuwon, V., Vaithanomsat, P., Chiemchaisri, C. and Chomsurin, C., 2007. Effects of ozonation process on lignin-derived compounds in pulp and paper mill effluents. *Journal of Hazardous Materials*, *142*(1-2), pp.250-257.
19. Mishra, M. and Thakur, I.S., 2010. Isolation and characterization of alkalotolerant bacteria and optimization of process parameters for decolorization and detoxification of pulp and paper mill effluent by Taguchi approach. *Biodegradation*, *21*(6), pp.967-978.
20. Mishra, S.B., Mishra, A.K. and Khan, M.A., 2010. Decolourization of pulp and paper mill effluents using heat-treated coal: a comparison with activated charcoal. *Environmental Chemistry Letters*, *8*(3), pp.231-235.
21. Morii, H., Nakamiya, K. and Kinoshita, S., 1995. Isolation of a lignin-decolorizing bacterium. *Journal of fermentation and Bioengineering*, *80*(3), pp.296-299.
22. Pedroza-Rodríguez, A.M. and Rodríguez-Vázquez, R., 2013. Optimization of C/N ratio and inducers for wastewater paper industry treatment using Trametes versicolor immobilized in bubble column reactor. *Journal of Mycology*, *2013*.
23. Rossi, S.P., Windschüttl, S., Matzkin, M.E., Rey-Ares, V., Terradas, C., Ponzio, R., Puigdomenech, E., Levalle, O., Calandra, R.S., Mayerhofer, A. and Frungieri, M.B., 2016. Reactive oxygen species (ROS) production triggered by prostaglandin D2 (PGD2) regulates lactate dehydrogenase (LDH) expression/activity in TM4 Sertoli cells. *Molecular and Cellular Endocrinology*, *434*, pp.154-165.
24. Raj, A., Reddy, M.K., Chandra, R., Purohit, H.J., Kapley, A., 2007. Biodegradation of kraft-lignin by Bacillus sp. isolated from sludge of pulp and paper mill. Biodegradation 18, 783–792.
25. Saha, R., Nandi, R. and Saha, B., 2011. Sources and toxicity of hexavalent chromium. *Journal of Coordination Chemistry*, *64*(10), pp.1782-1806.

26. Savant, D.V., Abdul-Rahman, R., Ranade, D.R., 2006. Anaerobic degradation of adsorbable organic halides (AOX) from pulp and paper industry wastewater. Bioresour Technol 97, 1092–1104.
27. Tewari, P.K., Batra, V.S., Balakrishnan, M., 2009. Efficient water use in industries: cases from the Indian agro-based pulp and paper mills. J. Environ. Manag. 90, 265e273.
28. Warren, G., Schultz, P., Bancroft, D., Bennett, K., Abbott, E.H. and Rogers, S., 1981. Mutagenicity of a series of hexacoordinate chromium (III) compounds. *Mutation Research/Genetic Toxicology*, *90*(2), pp.111-118.
29. Wang, C., Zhao, M., Lu, L., Wei, X., Li, T., 2010. Characterization of spore laccase from Bacillus subtilis WD23 and its use in dye decolorization. Afr J Biotechnol 10, 2186–2192.
30. Xu, R., Zhang, K., Liu, P., Han, H., Zhao, S., Kakade, A., Khan, A., Du, D. and Li, X., 2018. Lignin depolymerization and utilization by bacteria. *Bioresource technology*, *269*, pp.557-566.