BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, June 2024. Vol. **21**(2), p. 755-764

Strategies for Aerobic Decolorization and Detoxification of a Disperse Dye by an Isolate of Bacillus SP Pertaining to its Possible Correlation with Cod in Textile Effluent

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http://dx.doi.org/10.13005/bbra/3262

(Received: 11 January 2024; accepted: 20 May 2024)

The study was carried out to know significant involvement by uncovering the ability of native microbes isolated from the sample obtained from textile industry for decolorization and degradation of dyes and effluent through Isolated bacterial strains these were identified, through 16S rRNA Sequence analysis and confirmed the strains as, Bacillus cereus and Bacillus sp. The degraded metabolites were detected through HPLC (high performance liquid chromatography) analysis based on the retention time and the functional groups were detected through fourier transform infrared spectroscopy and the decolorization was carried through UV-visible spectroscopy by using the absorbance. The decolorization time required for decolorization was less in the consortia than the individual species these individual species were able to mineralize dyes. The SEM analysis revealed the consortium as tight clumps. The phytotoxicity results seed germination test with Vigna radiata reveals the positive results after the treatment through the consortium over the raw effluent.

Keywords: Aerobic Degradation; COD; Decolorization; Microbial Detoxification; Textile Effluent.

The upgradation in urbanization and industrialization had been paving towards the path of developed industries and contributing worldwide economy. The textile industries are showing effects on environment, as during textile processing, inefficiencies in dyeing results in huge dyestuff, as industry uses different dyes mixture to achieve various shades. In dye bath these concentrations are varying around 50-300 gram per litre along with a battery of chemicals as per Chaouch.[1] Ammayappan and Shakyawar[2], have been observed directly lost which find their way into floating water bodies showing the impact on the aquatic ecosystem, the textile effluent contains dyes and very complex chemicals which are the salts, metals, oxidising reducing agents and acids causing prolonged effect on water bodies [3].

As the physicochemical parameters of textile effluent exhibits high level of temperature, pH, with increasing concentration of COD, ultimately troubling the aquatic life, thus these parameters show adverse effect by absorbing light in receiving body which can cause the degradation [4]. Approximately million tons of wastewater is generated by textile industry. According to the study it is recalcitrant and toxic to receiving body, as revealed by different studies these dyes are mutagenic and carcinogenic [5]. The development

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of efficient treatment numerous studies was devoted which includes physicochemical treatment as, adsorption, coagulation, ion exchange, electrochemical precipitation and membrane process, [6] recently fenton reagent, photodegradation and sonochemical are showing effective results in decolorization. [7] However, these treatments are effective but also having some limitations can be point out as high cost, lack of selectivity, disposal problems, even generating toxic compounds after reaction [8].

Several studies have shown the interest in biotechnological approach in eco-efficient way. In general, the degradation through microbes is the effective, ecological sociability and lower sludge production to tackle wastewater. As bioremediation appears to be the promising alternative clean-up technology which involves microorganism for the degradation and removal of pollutants in a sustainable way which is inexpensive and eco-friendlier technology accepted worldwide. In 1989, 20 strains have been screened and three has resulted dye decolorization was reported by Ball [9]. However due to the toxicity impact of effluent, some studies have diluted the effluent and carried their work, and these require expensive additives [10] or pre-treatment like cooling and pH neutralization. The Bacteria has reported as efficient decolorization in pure culture Junnarkar [11] or in consortia [12], in addition contaminated metals and dyes have been remediate from soil and water.[13,14] Bacteria are subsequently able to mineralize aromatic amines aerobically.[15] In addition, fungi are also showing decolorization of textile dyes, but it requires lower pH for the enzyme activity and including longer retention time due to longer time requirement bacteria are preferred for decolourisation. The effective decolorization depends on adaptability and activity of microbes therefore, exploring them from the textile effluent and their identification and characterisation for decolorization still need valued to unravel their potential for rehabilitation of aquatic ecosystem. The key environmental issues associated with textile manufacturing are: Excessive use of water, effluent treatment, and disposal. consequently, the self-purification ability of the water body and even the conventional water treatment is hindered as around work has done to reduce keto groups and their sulphide bond of the indigo dye and in several contained bacteria can convert to molecule leading to the amine free.

Depend on the self-purification capability of the water is scrutinized moreover analysing their potential of dyes resistant bacteria isolated at laboratory scale. This work aims in understanding the potential of native bacteria in decolourisation for the development of their own strengths and observe degradation through HPLC and FTIR furthermore analysing phytotoxicity assay conducted for the toxicity level of treated and untreated effluent with respected to control.

MATERIALS AND METHODS

Sample collection and analysis

Textile effluent as a sample was collected from the industry and the conventional parameters as pH with pH meter model 64, the isolates were sent to NCCS, Pune. The culture was maintained on nutrient agar. All biochemicals were of analytical grade nutrient agar, agar- agar were from Hi-media. The pollution load of textile effluent was examined using the physico chemical parameters namely chemical oxygen demand (COD) Biological oxygen Demand (BOD) through standard estimation method, as these parameters serve as important criteria to measure pollution index.

Bacterial strains and culture from sample

The isolation was carried out by serial dilution of the effluent discharge from the textile industry through spread plate technique. After serial dilution sample was transferred on plated aseptically on the nutrient agar plate and incubated for 24 h at 30!. Pure culture was obtained through repeated streaking. Screening was done by grouping them with high growth rate of decolorization potential and identification based on gram staining and their morphological characteristics as their colony formation, motility, and pigmentation and furthermore identification was carried out by 16S rRNA nucleotide sequencing at NCCS, Pune. the nucleotide sequence was carried out in the NCBI server (http://www.ncbi.nlm.nih.gov). and alignment of the sequence was done with CLUSTAL-W program in MEGA software, after the removal of ambiguities the phylogenetic tree was constructed through neighbour-joining in Mega-11 software.

Decolourisation using microbes

The degrading cells were grown 8 hours for the exponential growth phase and afterwards obtained microbial culture were inoculated for 24 h in 50 ml culture tubes containing 10ml of Nutrient broth for the development of culture. After development of culture, 10 ml of isolates were added to Erlenmeyer flask containing 100 ml of textile effluent. The flask further was incubated to observe after time interval. 3 ml Aliquots was withdrawn for the separation of the cell mass, from the Erlenmeyer flask was and centrifuged at 5000 rpm for 15 min. several experiments were performed for the optimize decolorization with process condition. the decolourisation was observed using UV-Vis spectrophotometer using equation (1).

Decolorization=(Initial absorbance-Final absorbance)/(Initial absorbance)×100 ...(1)

Modelling the growth kinetics of microorganisms

The equation (2) for the determination of specific growth rate of consortia

$$\ln x/X_0 = \mu t \quad ...(2)$$

X=biomass concentration (g/L) at 't' time x_o is the initial concentration of biomass at time(t_0)
μ: is the specific growth rate(h^{-1})
Expression for growth yield(Y) is mentioned in equation (3),

$$dx/ds = Y \mu t \quad ...(3)$$

Rewriting equation as mentioned in equation (4),

$$X - X_0 = Y (S_0 - S) \quad ...(4)$$

Were,
X= is the biomass concentration
X_0 =is the initial biomass concentration
S= final concentration
S_0 =is the initial consortia COD concentration

Analytical analysis

As metabolites produced after the decolorization and degradation experiment sample were extracted with equal volume of ethyl acetate. The extract was dried over sodium sulfate and evaporated in the rotary evaporator. This sample was used for fourier transform infrared spectroscopy (FT-IR) and High-performance liquid chromatography (HPLC). The FTIR analysis of wastewater sample and degraded sample was caried out using IR-spectroscopy. The FTIR was done in the mid-range of 400-4000 cm^{-1} with the speed of 16-scan speed, as the sample adsorption and transmission are carried out as the molecule is going to stretching or bending and the transmitted light is detected on IR spectrum with transmittance and wavenumber. HPLC analysis was carried out in isocratic waters model no. 2690 and flow rate of 1.0ml for $10min^{-1}$, using -C18 guard column by using methanol as a mobile phase. the obtained data will be showing the degradation of sample. Scanning electron microscopy (SEM) is an analysis instrument which provides the high-resolution image through scanning of the surface Morphology.

Phytotoxicity

The study of toxicity assessment was carried obtained on control, treated and untreated effluent and using seed germination test with *Vigna radiata.* These beans were collected and sterilized with 0.2% w/v of $HgCl_2$. [16] For this assay petri dish were lined with the Whatman no.1 filter paper which was moistened with 5ml sample solution: 1) Control (tap water); Untreated textile effluent and treated effluent, were placed with 20 seeds in different petri dishes, and the germination percentage were calculated and was observed for 7 days. Toxicity was measured in terms of percent germination and the length of plumule and radicle. The germination index was calculated by formula as given by Rahman [17] mentioned in equation5,6 and 7.

Relaive seed germination=(number of seeds germination in treatment)/(number of seeds germination in control)×100 ...(5)

Relative root germination=(mean root length in treatment)/(mean root length in control)×100 ...(6)

Germination index=(Relative seed germination × Relative root germination)/100 ...(7)

RESULT AND DISCUSSION

Analysis of sample

As the collected sample was dark grey in colour with the pungent odour, as it consists of many chemicals. The pH of the sample was alkaline, due to presence of salts in the dyes processing. The temperature was high due to vatting operation in manufacturing process, characteristically decreasing the dissolved oxygen.[18] Higher BOD suggests a reduction of oxygen level and toxic nature, as the dye consist of ions concentration results in higher electrical conductivity. The elevating COD results show an increase in the toxicity level due to the dye-fixing agent. As these higher values get reduced after treatment. The

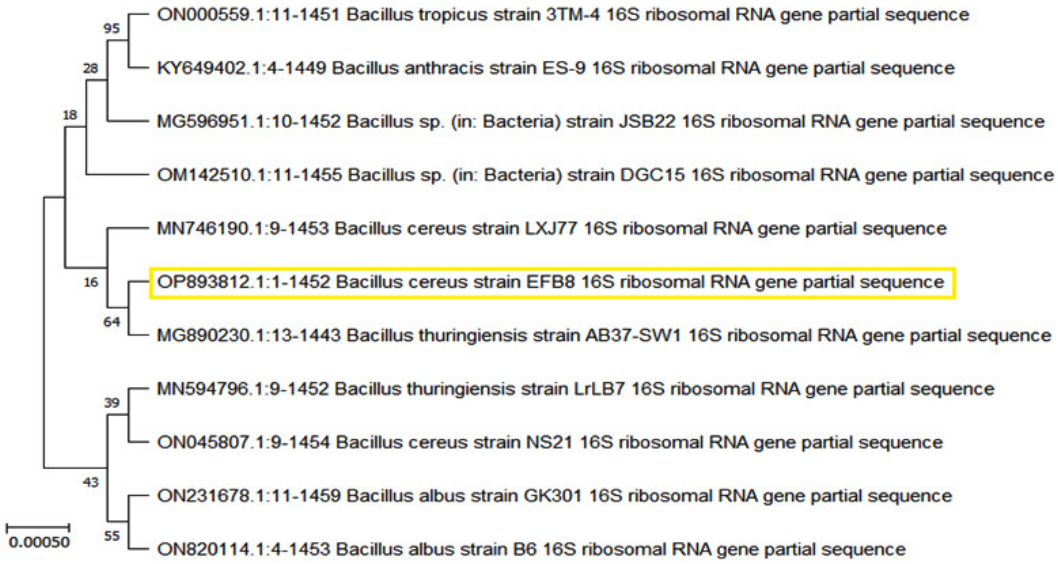

Fig. 1. The phylogenetic relationships of *Bacillus cereus* EFB8

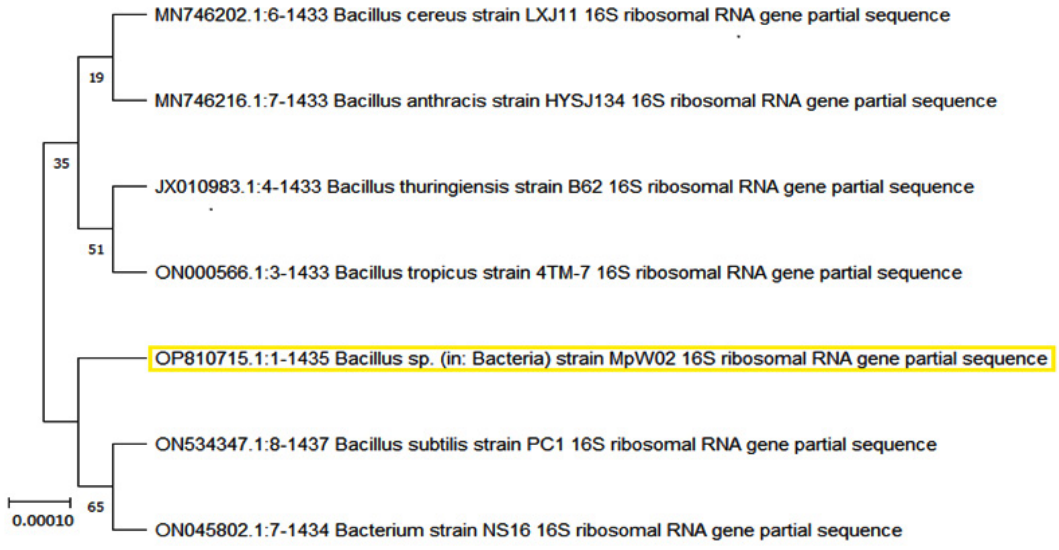

Fig. 2. *Bacillus sp.*MpW02.the bootstrap is shown on the nodes

strength of microbes has shown efficient results as the characteristics before and after the treatment with the consortia was recorded.

Evaluation of COD at various time intervals has shown that there was increasing in percent of decolorization with the decreasing COD value. As Observed before treatment the COD was 3990 mg/l (untreated) the sample was untreated as the sample was treated with the consortia there was reduced to 1292 mg/L overall results were showing effective reduction by the action of consortia. As well with COD reduction there was efficient decrease in the BOD at 800 to 310 mg/L. after treating it with consortia nearby was significant

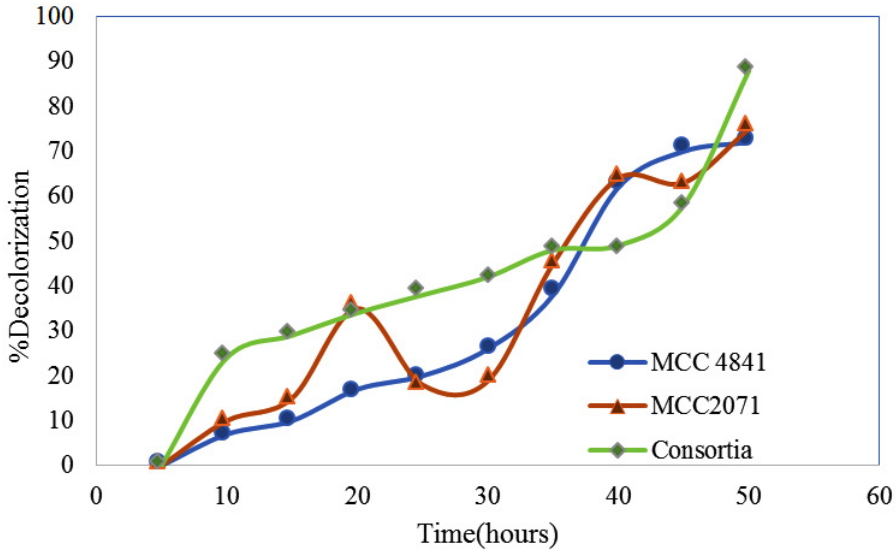

Fig. 3. Showing Percentage of Decolorization

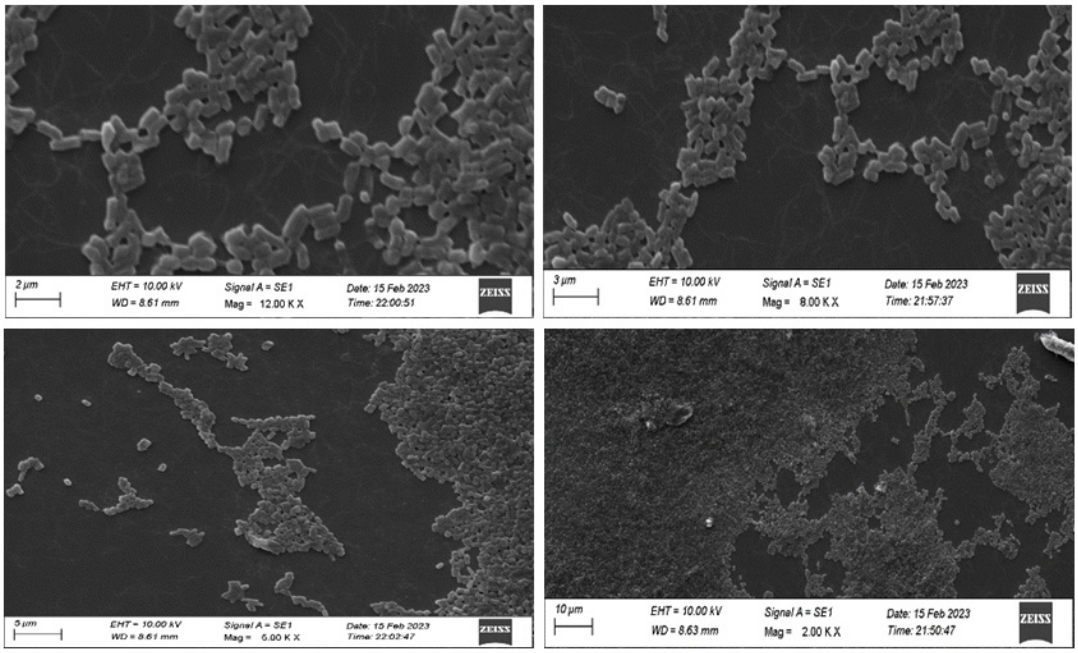

Fig. 4. Morphological structure of the strains analysed through scanning electron microscopy

decrease in BOD. As the reduction in COD and BOD after treatment shows removal of organic load from the sample.

Isolation and Screening of bacterial strains from effluent

The textile dye revealed that sample was containing mixture of azo dyes. The bacteria capable of decolorization was isolated from textile industrial effluent by spread plate technique. Two bacterial strains as MCC 4841 and MCC 2071 both of this strain has shown the effective degradation. It found to belong to *Bacillus cereus and Bacillus sp*. These were used to decolorize the effluent which were isolated from the textile effluent. Their identification was carried out by16r RNA gene sequencing analysis with 700 bp and the results are shown, the phylogenetic tree is built with the help of NCBI-BLAST-n (http://blast.ncbi.nlm.nih.gov/) and as shown in figure 1 and figure 2.

Screening of bacteria against decolorization effluent

The strains of MCC 4841 and MCC 2071 were tested individually and in consortia for decolorization of the sample under static conditions through microbial metabolic activity not providing any physical conditions in complete aerobic medium.

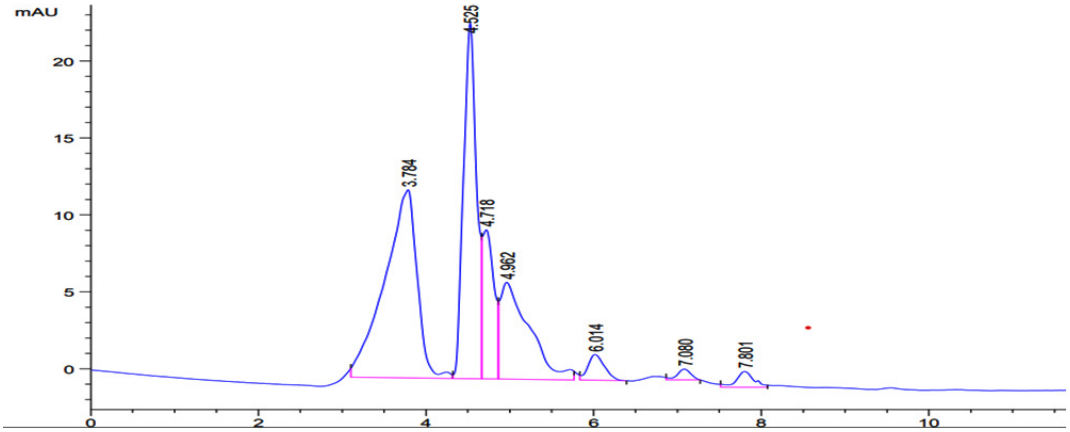

Fig. 5. HPLC spectra of textile after treatment with the consortia

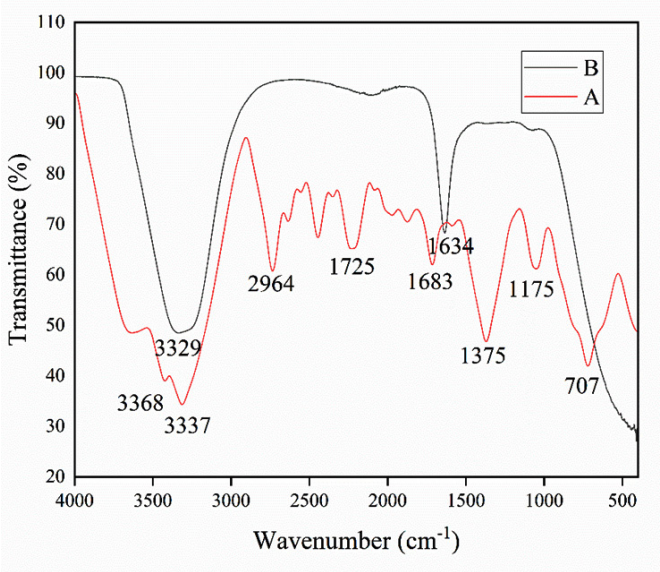

Fig. 6. IR-Spectra analysis of (A) Untreated sample and (B) Treated sample

Individually, MCC4241 as *B. cereus* and *B. sp.* MCC 2071 can form a cooperation and can benefit in many ways. could decolourise the sample to 72% and MCC 2071 has shown about 75% decolorization respectively as shown in figure 4 and the consortia have shown enhanced results as compared to individual strains as it found 88% decolourized within 48h. As mentioned by Chen, [20]some of the bacteria are present in activated sludge, either they are degrading or decolourize, or some enhance this activity by producing metabolites as these microbes work in a mutual way through which one can reach the goal of minimizing the pollutant. According to Chen study also revealed that massive addition to induce the degrading activity which leads to undesirable results which affect their equilibrium, [20] so there should be well-balanced ratio of these species. Bouchez says that balance must be maintained for the removal of pollutant and to treat effluent in effective manner.[21] MCC4241 as *B. cereus* and *B. sp.* MCC 2071 to analyse its morphological structure scanning electron microscopy (SEM) analysis has studied, the results shown in figure 4 reveal that strains are of rod-shaped cells.

Growth kinetics

The dry weight of the biomass and the yield coefficient was observed after treating of sample using consortia of MCC4021 and MCC2071. The specific growth rate was 0.13 h^{-1} and the yield coefficient of 3.12 mg dry weight of the biomass. The literature reported the specific growth rate varying from 0.001 to 0.004 h^{-1} and yield coefficient varying from 0.35 to 0.65 mg of dry weight of biomass

Biodegradation analysis

The decolorized and degraded sample was analyzed under static conditions. Analytical analysis through UV-vis spectra, FTIR and HPLC were employed for biodegradation of the treated and untreated sample. UV-vis optical density was recorded and plotted on graph in the relation with time, the significant decreased through decreasing peak shown by consortium as mentioned in figure 5. The HPLC elution profile of biodegradation of the sample shown significant peaks at 3.7, 4.5, 4.7 ,4.9, 6.0, 7.0, 7.8. which confirms the degradation of sample as shown in figure 5 Showing the HPLC results.

Fig. 7. Phytotoxicity test: analysis by *Vigna radiata* seeds in control treated and untreated sample

The Results of FTIR analysis of sample(A) as untreated and sample(B) as treated attained after untreated spectrum of the effluent showed presence of centered peak at 3368cm^{-1} is peak of 2-methyl -1,5 pentanediamide, -NH stretch, hydrogen bonded, primary amine coupled doublet: asymmetric. O-H stretch at 3337cm^{-1}is the intermolecular hydrogen bonding, due to 2-Methyl-1-butanol. The 2964cm^{-1} is the C-CO-C stretch indicates methylene.1725cm^{-1} in this region the small amount of unbounded enolic form is responsible for the centring due to the keto group. at 1683cm^{-1}for stretch due to C=O, the ring due to C-C bend. The C=C stretching vibration is due to hydrocarbon molecule at the region 1634cm-1 the conjugated alkenes in the treated sample. At 1375cm^{-1} near this region is absorption band arising from symmetrical bending of methyl C-H bonds which is highly stable group the isopropyl group almost equal intensity at 1385-1380cm-1. 1175cm^{-1} is the methyl ester long chain fatty acids near band which is the strongest bond. 707cm^{-1} The C-H bending vibration of broad absorption in the 700-610cm-1 region lead by alkynes. This stretching and deformation bond clearly indicates there is degradation of the effluent, here in analysis of peaks of sample B resulted confirm the biotransformation of sample through microbial action.

Phytotoxicity test

As the dye stuff and heavy metals once reach to the environment are harsh to it, Phytotoxicity test of effluent for before and after treatment was monitored by growing *Vigna radiata* seeds, to assess the feasibility of sample before and after treatment phytotoxicity test was performed the experiment results showed in figure.7 this reveals there is significant reduction in the growth of shoot and root length, there was quite an elongation in the root and shoot in the starting days and afterward, the growth was slowed down in the untreated sample. There was at least maximum growth was seen in treated sample as compared to the untreated. the maximum germination percentage in tap water (control sample) up to 83% and the treated sample results were 74% which was nominal toxicity of dye and the parent effluent showed 38% germination which showed that the presence of toxic components which has inhibited their germination, However, our study showed that number of parameters were comparatively high in untreated effluent, which have fatal consequences for the germination of seed. furthermore, it is widely accepted that the discharge from industry discharge shows degradation of soil, impact on productivity, soil and environment.

CONCLUSION

The uniqueness of the present study is to construct the native microbe which were able to decolorize the effluent and degrade up to removal efficiency and to reduce the preliminary processes at laboratory for the cheap, easy, and efficient alternative for the reduction of preliminary isolates was the preliminary intention, Result established the best yield were observed through microbial consortia were working more efficiently in reduction of color and COD than the individual. The isolation of microbes as from sample were *Bacillus cereus* and *Bacillus sp.* which work effectively. The Phytotoxicity assessment revealed that the real effluent was showing higher levels of toxicity than the after-treatment product. The HPLC and FTIR analyses of the produced metabolites after degradation of sample showed the presence of detoxification to the lesser molecules due the action led by microbes while led to mineralization. In the direction of degrade these dyes in the environment is a critical challenge and detailed mechanism at the industry level.

ACKNOWLEDGEMENT

Sincere thanks to Dr. Babasaheb Ambedkar Research and Training Institute for Providing, Dr. Babasaheb Ambedkar National Research Fellowship 2020 funded by Department of Social Justice and Special Assistance, Government of Maharashtra, India for financial support as the award letter number is BARTI/ Fellowship/BANRF-2020/21-22/850, and Director, School of Environmental and Earth Sciences, Kavayitri Bahinabai Chaudhari North Maharashtra University, Jalgaon for providing laboratory facility.

Conflict of Interest

There is no conflict of interest.

Funding Sources

Dr. Babasaheb Ambedkar National Research Fellowship 2020 funded by Department of

Social Justice and Special Assistance, Government of Maharashtra, India for financial support as the award letter number is BARTI/Fellowship/BANRF-2020/21-22/850

Authors' Contribution

Not applicable.

Data Availability Statement

Not applicable.

Ethics Approval Statement

Not applicable.

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