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Decolourization of Two Industrial Dyes by Bacteria from Paper and Pulp Mill Effluents

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Abstract

The effluents of paper and pulp industries are the major sources of water pollution, threatening the existence of aquatic biodiversity. So, their treatment before discharge is very crucial and biodegradation where microorganisms break down the azo bonds to form its nontoxic basic element is the most effective way. Our research work deals with the physicochemical characterization of the concerned effluent, isolation of indigenous bacterial strain and evaluation of their decolorizing efficiency. Decolorization assay was done spectrophotometrically in Mineral salt medium modified with 0.005% of the tested dyes viz. Brilliant green and Methyl violet after regular interval of incubation period (3, 5 and 7 days). All the collected effluent samples were found to characterize by high BOD, COD, TSS and TDS values. Five potential isolates viz. Alcaligenes eutrophus, Micrococcus luteus, Bacillus licheniformis, Bacillus subtilis, and Pseudomonas syringae were identified and their decolourizing capability were evaluated. Alcaligenes eutrophus Micrococcus luteus. and Pseudomonas syringae were found to decolourize around 80% of the Brilliant Green after 5 days of incubation whereas Bacillus subtilis and Bacillus licheniformis causes slightly more than 60% decolourization after the same incubation period. Bacillus licheniformis was found to be the only isolate capable of decolourizing Methyl violet with maximum of 75% after 7 days of incubation. These findings reveal the existence of indigenous dye decolourizer and signify their implementation in biotreatment of the tested dyes.

Keywords: Water pollution, industrial effluent, dyes, biotreatment.

Introduction

Modern life with increased urbanization and industrialization inevitably led to an anthropogenic impact on the biosphere through the production of a huge amount of wastes. Dyeing process of textile, paper, leather and cosmetic industries resulting the release of upto 50% of the dye as effluent regarded as one of the principal source of water pollution where its annual production is 7×10^5 metric tons[1-3]. However, these toxic dyes are unfortunately designed to be resistant against many degradation processes and they itself and even in some cases their breakdown products show carcinogenic and cytotoxic effects[4]. Moreover, intense colour of dyes limits light penetration and oxygen transfer through the aquatic surfaces resulting photosynthetic interference of phytoplankton[5,6]. Increased BOD and COD values of the dye effluent make it difficult to degrade and create a toxic environment for aquatic biodiversity[7]. So, their treatment before releasing to the environment is so vital to minimize water pollution. Among different treatment processes, biotreatment has regarded the most efficient way in comparison with conventional physicochemical processes having drawbacks of being expensive, complex structural setup, high chemical and power requirement and production of sludge[2]. In many cases those conventional processes are not suitable enough for complete degradation of dyes to CO_2[8,9] . Biotechnological approaches are replacing those conventional treatment processes since many microorganisms such as *Bacillus, Micrococcus, Pseudomonas*, *Aeromonas* has long been known to degrade various toxic azo dyes consisting phenylamine benzenediazonium chloride or phenol[10-12]. Furthermore, bioremediation involving the application of those bacteria are cheap, structurally simple, environment friendly and produce less sludge than other approaches[13-15]. For efficient biodegradation it is also important to select microorganisms capable of degrading that dye at concentration present in the effluent. Thus, our study mainly deals with the isolation, identification of strong dye decolourizer from paper and pulp industry effluent which could be used for the bioremediation of these dyes.

Material and Methods

Sample: Effluent samples from four different discharge points (Bleaching chemical drain, Recovery and pulp mill drain, combined drain and downstream river water) of Karnaphuli Paper Mills Ltd, Chittagong, Bangladesh were collected aseptically in sterile glass bottles. After collection, temperature, pH, color, date and location of the samples were recorded and samples were brought to the laboratory immediately in a cooler box. In laboratory, physicochemical properties of the samplessuch asBOD, COD, TDS and TSS of the samples were evaluated.

Dyes and Media: Dyes used in this experiment (Methyl Violet

and Brilliant Green) were generous gift from the mentioned industry. Nutrient agar media (HiMedia Laboratories) was used for the enumeration, isolation and purification of bacteria from collected effluents. For decolourization assay, Mineral salt media (MSM) possessing with NaH_2PO_4 (0.235%), $MgSO_4.7H_2O$(0.007%), $CaCl_2$(0.014%) and $FeCl_3.6H_2O$ (0.0001%) of pH 6.5 modified with 0.005% of respective dye was used.

Plating: Immediately after sample collection, bacterial number was enumerated by applying dilution plate method. After enumeration, colonies with distinct character were isolated, purified and screened to figure out the potential dye decolourizer.

Screening: Screening for the potential isolates was done by inoculating their juvenile culture in Nutrient Broth media modified with 0.001% of the respective dye. After incubation at 37°C with frequent monitoring for 24 to 72 hours, positive test tubes were identified by comparing them with an uninoculated one serving as control. After that, isolates showing positive result were selected, purified and preserved for further studies.

Identification: The isolates showing positive screening test were examined for their morphological properties, such as size, shape, cell arrangement and staining properties. Cultural properties including form, colour, elevation, margin, surface of colonies on nutrient agar plate and slant were also recorded. Physiological and biochemical characteristics of the isolates were evaluated by Voges–proskauer, methyl red, indole, catalase, oxidase, urease, citrate utilization, nitrate reduction, gelatin liquefaction and H_2S production tests. The ability of the organisms in fermenting a number of sugars including glucose, fructose, sucrose, arabinose, mannose, rhamnose, galactose, maltose and lactose were also performed. The isolates were identified up to species based on comparative analysis of the observed characteristics with the standard description of bacterial strains in Bergey's Manual of Determinative Bacteriology[16].

Decolourization assay: Decolourization assays of the potent isolates were done in Mineral salt medium (MSM) modified with 0.005% of the two tested dyes. Inoculum was developed in nutrient broth medium by incubating for 20 hours at 30°C and 200 rpm in an orbital shaker incubator (Model SI50, Stuart Scientific, UK). The Decolourization test was then performed by adding 5% (v/v) of the inoculum in Erlenmeyer flasks having 50 ml decolourization media and pH adjusted to 6.5 with 0.1M phosphate buffer. After incubating at 30°C and 200 rpm, broth cultures were withdrawn at specified interval (3^{rd}, 5^{th} and 7^{th} days), centrifuged at 10,000rpm for 15 min at 10°C . Than the supernatants were collected to obtain their optical density (OD) to be measured at 520nm under visible light in spectrophotometer (UV-VIS RS spectrophotometer, LaboMed. Inc.). OD of the control (uninoculated) flasks were also measured. From these OD values dye residual dye concentrations were obtained from Standard curve. Finally, rate of decolourization was calculated as percent of decolourization (%) = $(A_i - A_t) / A_i \times 100$, where A_i was the concentration of the initial dye solution and A_t was the concentration at cultivation time (3, 5 and 7 days). Each test was performed triplicate and mean values expressed as result.

Results and Discussion

Paper dye effluents are toxic threat to biodiversity: The temperature of the collected samples were found within the range of growth temperature suitable for mesophilic microorganisms (30°C to 39°C) where the pH were found higher ranging from 7.5 to 11.2. Furthermore, BOD values of the samples was found to range from 450 to 720 mg/L while COD values found to vary from 1050 to 1500 mg/L. In all cases, BOD and COD ratio was found more than 0.2 which is highly toxic to biodiversity and difficult to remediate by conventional treatment processes. Additionally, collected samples were found to have extreme TDS varied from 11023 to 11500 mg/L and corresponding TSS were 12500 to 13000 mg/L. High values of all these physicochemical parameters of sample quality indicator necessitates its treatment before discharge into the environment.

Substantial number of bacteria present in dye effluent: A significant number of bacteria was found to present in the collected samples ranging from 18.75×10^9 to 11.8×10^{15} cfu/ml by applying dilution plate method where maximum number was found to present in recovery and pulp mill effluent. Though the dye effluent is toxic to most of the living entities, the presence of such a notable amount of bacteria indicates the ability of some members of microbial community capable of using them as a source of nutrient.

Paper dyes can be degraded by bacteria: From the enumerated plates, 24 bacterial colonies were isolated on the basis of their distinct colony characteristics like form, colour, elevation, margin, surface, etc. These bacteria were then screened to figure out potential decolourizer of Methyl Violet and Brilliant Green in a medium having 0.001% of the respective dye. Through the screening process a total of 5 isolates were selected as potential dye decolourizer and preserved for further study.

Identification of the selected isolates: Five potential isolates were identified on the basis of their morphological, cultural, microscopic and biochemical characteristics including size, shape, form of the bacterial cells, presence or absence of spores, acid fastness, gram reaction, IMViC test, H_2S production, nitrate reduction, different carbohydrates fermentation etc. All these characteristics were analyzed with the standard description given in "Bergey's Manual of Determinative Bacteriology" [16] and they were identified as *Alcaligenes eutrophusMicrococcus luteus, Bacillus licheniformis, Bacillus subtilis,* and *Pseudomonas syringae*.

Decolourization assay of the isolates: Isolates showing positive screening test were subjected to decolorization assay against two industrial dyes namely Brilliant green (triphenylmethane dye) and Methyl violet (azo dye) under shaking conditions. Decolourizing ability of the isolates were determined spectrophotomertically. The selected isolates showed different patterns of decolourization where some are rapid and others are comparatively slow decolourizer.

Among the isolates, *Alcaligenes eutrophus, Pseudomonas syringae, Micrococcus luteus* showed highest extent of Brilliant green decolorization. They decolourized around 80% of the initial dye concentration where the fastest onset displayed by *Pseudomonas syringae* and *A. eutrophus* decolourizing slightly more than 75% on the third day. Though we tested for 7 days of incubation for all decolourization assay, in this case highest decolourization achieved after 5 days of incubation. It may be due to the strong metabolic activity of the young bacterial cells. Other isolates also exhibited 60% efficiency that confirmed the potential of these organisms for bioremediation of the hazardous tested dyes.

spectrophotometrically after 3, 5 and 7 days ofincubation by using the formula: Decolourization (%) = (Ai– At) / Ai × 100, where Ai denotes absorbance of the initialdye solution and At denotes absorbance at cultivation time.Above data is the result of the triplicate experiments.Here, * = P <0.05, ** = P< 0.01 and *** = P< 0.001, P =significance level.

In case of methyl violet decolorization no isolate other than *Bacillus licheniformis* was found to cause significant decolourization. At the initial stage, decolourization was seemed slow where only 11% decolourization occurs after 3 days of incubation. But, *Bacillus licheniformis* gradually decolourize this dye upto 75% after 7 days of incubation while decolourizing 50% within 5 days of incubation period. Slow initial decolourization of this bacteria might be occur for taking time to acclimatize with the toxic dye environment.

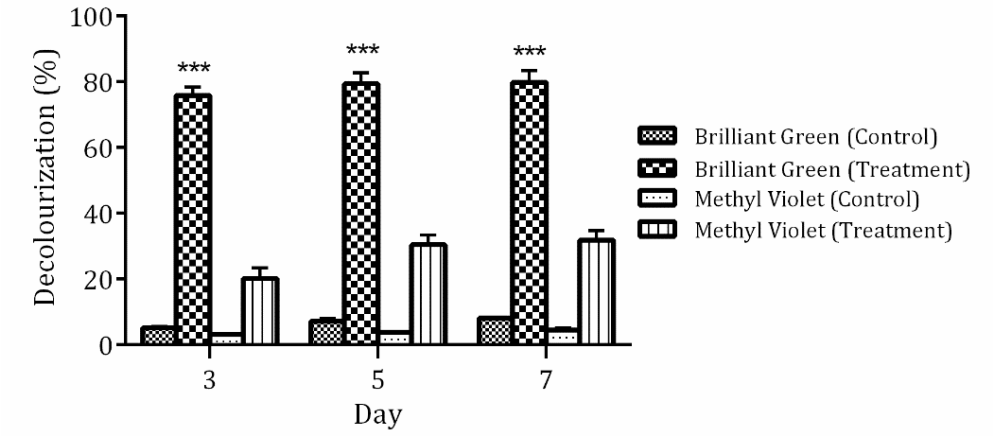

Figure-1
Decolourization of Brilliant Green and Methyl violet by *Alcaligenes eutrophus*. The assay was performed in modified mineral saltmedium having 0.005% of dye, pH 6.5. Decolourization (%) were measured

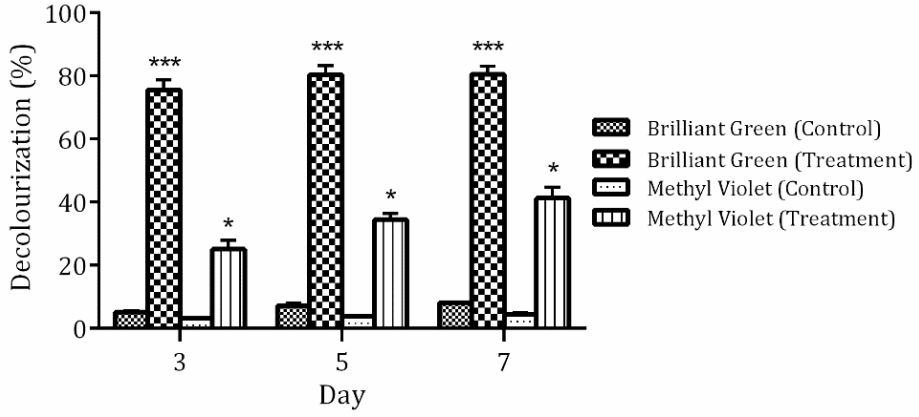

Figure-2
Decolourization of Brilliant Green and Methyl violet by *Pseudomonas syringae*. The assay was performed by following the method mentioned in figure 1. Above data is the result of the triplicate experiments. Here, * = P <0.05, ** = P< 0.01 and *** = P< 0.001, P =significance level

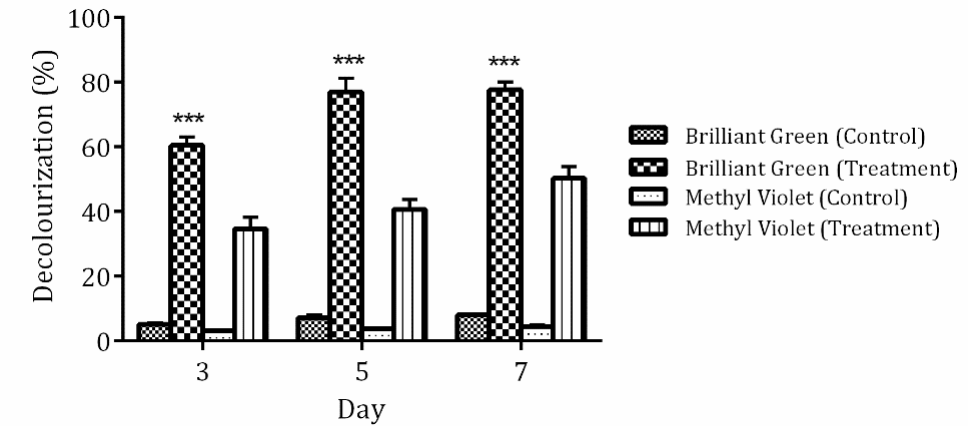

Figure-3

Decolourization of Brilliant Green and Methyl violet by *Micrococcus luteus*. The assay was performed by following the method mentioned in figure 1. Above data is the result of triplicate experiments. Here, * = P <0.05, ** = P< 0.01 and *** = P< 0.001, P =significance level

In other cases, percent decolourization was found to range within 30 – 50 after 7 days of incubation. All potential Brilliant Green decolourizers not significantly decolourize Methyl violet where *M. luteus*, *P. syringe* and *A. eutrophus* decolorized 55%, 41% and 31% after 7 days of incubation.

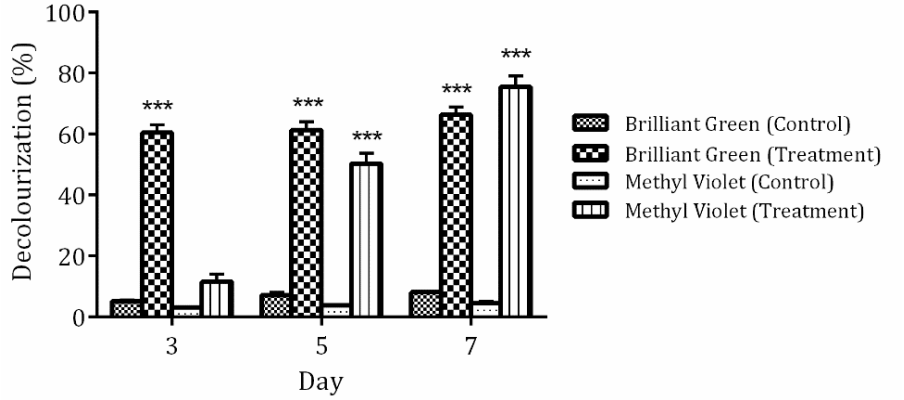

Figure-4

Decolourization of Brilliant Green and Methyl violet by *Bacillus licheniformis*. The assay was performed by following the method mentioned in figure 1. Above data is the result of triplicate experiments.Here, * = P <0.05, ** = P< 0.01 and *** = P< 0.001, P =significance level.

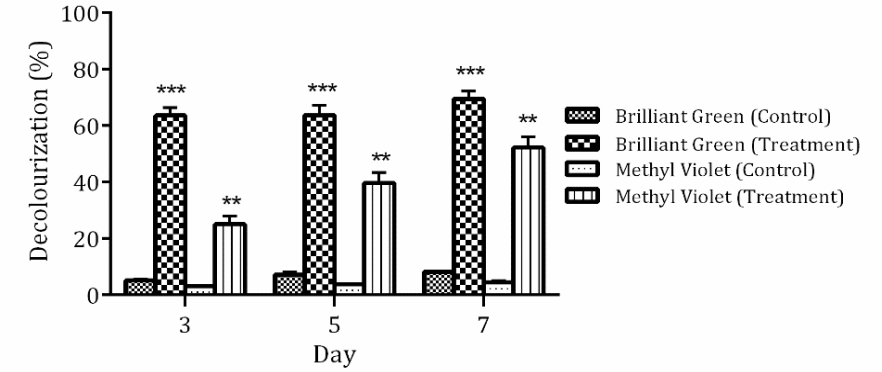

Figure-5

Decolourization of Brilliant Green and Methyl violet by *Bacillus subtilis*. The assay was performed by following the method mentioned in figure 1. Above data is the result of triplicate experiments.Here, * = P <0.05, ** = P< 0.01 and *** = P< 0.001, P =significance level

Some previous experiments studied biodegradation of methyl violet by fenton process finding 97% decolourization of Methyl violet[17]. In our experiment, *Bacillus licheniformis*; the only potential isolate in this experiment degrading Methyl violet at an extent of 75%. This isolate could be used in ETP of paper effluent being less expensive than fenton process to remediate this dye. The decolorization of azo dyes via reduction of azo bond has been shown *by Aeromonas hydrophila, Proteus vulgaris, Providencia rettgeri* by several previous experiments[18] as well as *Bacteroides* sp., *Eubacterium* sp., and *Clostridium* sp. *Proteus vulgaris* and *Streptococcus faecalis*[19,20].

Conclusion

This study revealed that the native isolated bacteria *Alcaligenes eutrophus Micrococcus luteus,Bacillus licheniformis, Bacillus subtilis, Pseudomonas syringae* are more potential bacterial isolates which would effectively use in treatment strategy of dye decolorizaton and mineralization of the paper and pulp mill dye effluent. Their proper utilization in effluent treatment plant would certainly play a significant role in remediating those paper dyes and thus the pollution from this effluent could be minimized. Further research on their enzyme property, process parameter optimization and molecular analysis of degradation process would make them more efficient degrader.

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