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Biodegradation of Textile Azo Dyes by Bacteria Isolated from Dyeing Industry Effluent

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Abstract

Water pollution caused by industrial effluent discharges has become an alarming trend worldwide, while textile industries are considered as the most polluting among all others. In recent years, bio-treatment took attraction in removing the unwanted colour and toxicity of textile effluents than other conventional treatment processes. The present study concentrates in the isolation and identification of indigenous bacteria from textile dye effluent and evaluation of their ability to decolourize dyes. The decolourizing activity was measured spectrophotometrically after incubation of the isolates for 3, 5 and 7 days in mineral salt medium modified with 0.05% of respective Novacron dye, viz orange W3R, red FNR, yellow FN2R, blue FNR or navy WB. Three bacterial isolates exhibiting strong decolourizing activity were identified up to species as Micrococcus luteus, Listeria denitrificans and Nocardia atlantica. All the bacteria exhibited maximum decolourizing activity after 7 days of incubation with little deviation. The bacterium Micrococcus luteus caused 60% decolourization of yellow FN2R and navy WB, and 85-90% of orange W3R, red FNR and blue FNR. Likewise, Listeria denitrificans decolourized 70-80% of Blue FNR, Orange W3R, Red FNR and Navy WB. In contrast, the bacterium caused no significant decolourization of yellow FN2R. Notably, Nocardia atlantica caused almost complete decolourization of Blue FNR and Red FNR, while at least 80% of other dyes tested. This study thus reveals that some bacteria inhabit in textile effluent whereby utilize the dyes as their source of energy and nutrition, and imply their importance in treatment of industrial effluents.

Keyword: Water pollution, industrial effluent, Azo dye, Bioremediation.

Introduction

Rapidity of industrialization and urbanization around the world has lead to the recognition and understanding of relationship between environmental pollution and public health[1]. While, the pollutions triggered by the human activities become the top most challenge for modern civilization[2-4]. Among the most concerned environmental pollutions that threatening our biodiversity, water pollution is a major one where effluents from dye-based industries serve as principal source[5]. Textile industries consume a huge volume of azo dyes while up to 50% of dyes find its ultimate way in the water as effluent[6]. The structures of azo dyes consists coupling of diazotized amine with either an amine or a phenol and also contain azo linkage(s)[7]. Most of these dyes are potentially toxic to aquatic life[8] and some are even carcinogenic and mutagenic to humans[9]. Furthermore, colour of the dyestuff interrupts the aquatic environment by reducing light penetration, gas solubility and interference of phytoplankton's photosynthesis[10]. Textile azo dyes are sometimes found difficult to degrade completely, and the conventional physico-chemical treatment processes are not always suitable enough for their complete degradation and conversion to CO_2[11,12]. Methods like chemical precipitation, adsorption, and flocculation have substantial disadvantages, which include complex structural set-up, huge chemical and power consumption and formation of a large volume of sludge[13]. The physico-chemical sludge is highly toxic and troublesome to safe disposal[13]. In contrast, remediation of dyeing industry effluent by using microorganisms has proved to be the best solution[14] since numerous bacterial species including *Bacillus, Pseudomonas, Enterobacter, Halobacter,* and *Aeromonas* have been reported to exhibit tremendous capability to decolourize and detoxify a wide range of azo dyes composed of phenylamine, benzenediazonium chloride or phenol[15-17]. In most cases, bacteria disintegrate azo bonds of the dyes, which result in the formation of colourless amines and subsequently simpler compounds[18]. In recent days, the use of technologies based on bioremediation has got much attention for the treatment of textile dye effluent because of simple structural set-up, low cost, easy to operate, less sludge volume, environmental benignity, and wider application[19-21]. Apparently, the development of novel biological decolourization system consisting one or more acclimatized microorganisms in habitat concentration is urgently needed for the effective clean up of the excess dyes in effluent. This study attempts to isolate and identify bacterial strains possessing strong dye-decolourizing capacity, which can be potential candidate agent for the remediation of textile dye effluent.

Material and Methods

Azo dye: Textile azo dyes of Novacron family, *viz.*, orange W3R, red FNR, yellow FN2R, blue FNR and navy WB were

generous gift from Qualitex Industries (BD) Ltd, Export Processing Zone, Chittagong, Bangladesh.

Sample: Dye effluent samples were collected from 3 discharge points of Qualitex Industries (BD) Ltd., CEPZ, Chittagong, Bangladesh. The Samples were brought to the laboratory on an ice pack in a cooler box and stored at 4^{o}C.

Culture media: Nutrient agar media (HiMedia Laboratories) was used for the enumeration and isolation of bacteria from the dye-effluent. Mineral salt media possessing 0.235% NaH_2PO_4, 0.007% $MgSO_4.7H_2O$, 0.014% $CaCl_2$ and 0.0001% $FeCl_3.6H_2O$ have pH 6.5 and modified with 0.05% of respective dye was used for the decolourization test.

Screening of Dye-Decolourizing Bacteria: Effluent samples were enriched by co-incubating in nutrient broth containing 0.01% of each dye at 35^{o}C for 24 hours. A minute volume of each enrichment culture was plated onto nutrient agar medium supplemented with 0.01% of respective dye. Following incubation at 35^{o}C for 24 hours, the resulting bacterial colonies exhibiting clear zone around them were isolated on the basis of colony morphology and dimension of clear zone.

Identification of selected isolates: The selected isolates 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[22].

Dye Decolourization Assay: Bacterial Inoculum was prepared by incubating few drops of bacterial suspension in nutrient broth in an orbital incubator (Model SI50, Stuart Scientific, UK), shaking continuously at 200 RPM for 20 hours at 30^{o}C. The test was initiated by incubating 5% (v/v) of the inoculum to the decolourization medium at 30^{o}C and 200 RPM. At defined intervals of 3rd, 5th and 7th day, the culture was withdrawn, centrifuged at 10,000g and 10^{o}C for 15 min, and the supernatant was examined for absorbance at 530 nm under visible light in a spectrophotometer (UV-VIS RS spectrophotometer, LaboMed. Inc.). The extent of decolourization was expressed as percent (%) decolourization and estimated as $(A_i - A_t) / A_i \times 100$, where initial absorbance of the dye solution and absorbance at cultivation time denoted by A_i and A_t respectively.

Results and Discussion

Indigenous Bacteria of Textile Dye Effluent: It has long been reported that bacteria inhabits in industrial effluents utilizing its constituents as their source of energy. Likewise, the textile dye effluents examined in this study was appeared to harbor a diverse community of microorganisms. In this study, the average bacterial load was found 2.8×10^7 cfu/ml as determined by total viable count performed by serial dilution of the effluent and subsequent inoculation onto nutrient agar medium. This finding is similar to some other previous studies[23]. These bacteria are of indigenous type and the dye effluent serves as their source of nutrients. A total of 11 bacterial strains were isolated from textile dye effluents based on their distinct colony characteristics like form, colour, elevation, margin, surface, etc. It thus provides evidence that textile dye effluent harbor a wide range of bacterial species that may even degrade the dyes to obtain their essential elements.

Bacteria Capable of Decolourizing Azo Dye: Screening of the bacterial isolates was performed to figure out the isolate capable of degrading textile azo dyes of Novacron family, namely, orange W3R, red FNR, yellow FN2R, blue FNR and navy WB in mineral salt media containing 0.05% of respective dye. Notably, only 3 bacterial strains capable of decolourizing the majority of dyes up to 60% were screened and considered as potential candidates.

Identification of Dye Decolourizing Bacteria: The bacterial strains exhibiting strong decolourizing activity was investigated for their morphological, cultural, physiological and biochemical features. After scrutinizing the properties with that described in Bergey's Manual, the bacterial strains were identified as *Micrococcus luteus*, *Listeria denitrificans* and *Nocardia atlantica.*

Evaluation of Dye Decolourization Rate: Among the dyes, Novacron Blue FNR was more than 80% decolourized by all the isolates and almost completely decolourized by the isolate *N. atlantica* (figure 1). This is also true in case of Novacron Orange W3R decolourization where *Micrococcus luteus* was the highest decolourizer (figure 2). In each time *M. luteus* decolourized 70% of the dye within 3 days of incubation. It might be due to its short lag phase in this toxic dye environment. In contrast, *L. denitrificans* took comparatively long time to acclimate, causing only around 20% decolourization within same incubation period. But, surprisingly the decolourization (%) achieved after 5 days of incubation was found as the double of that achieved after 3 days of incubation.

Novacron Red FNR and Navy WB was also well decolourized by all the isolates and in both cases *N. atlantica* was the best decolourizer causing impressive 95.7% and 85% decolourization respectively. Moreover, *M. luteus* after decolourizing 75% of Novacron Red FNR within 3 days of

incubation, reached a decolourization value of 89% after 7 days of incubation. Like other experiment, *Listeria denitrificans* again showed a long lag phase. But after being well adapted, this bacterium performed rapid degradation and finally caused more than 70% decolourization (figure 3 and 4).

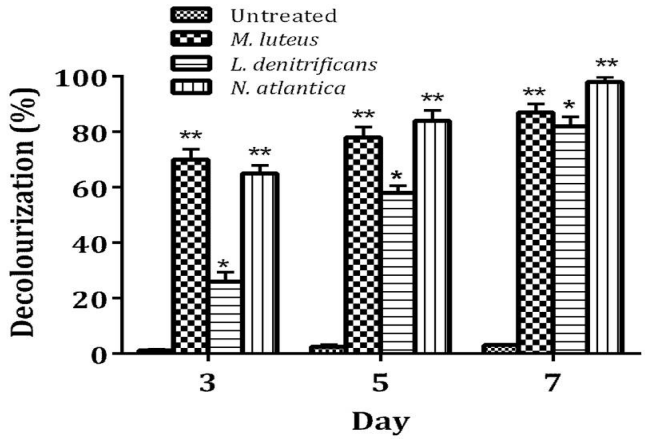

Figure-1

Decolourization of Novacron Blue FNR by bacterial isolates, The assay was performed by using isolates *M. luteus, L. denitrificans and N. atlantica* in modified mineral salt medium with 0.05% of dye and pH 6.5. Decolourization was measured spectrophotometrically after 3, 5 and 7 days of incubation by using the formula: Decolourization (%) = (A_i – A_t) / A_i × 100, where A_i denotes absorbance of the initial dye solution and A_t denotes absorbance at cultivation time. The data is representative of three independent experiments. Here, * = P <0.05, ** = P< 0.01 and *** = P< 0.001, P = significance level.

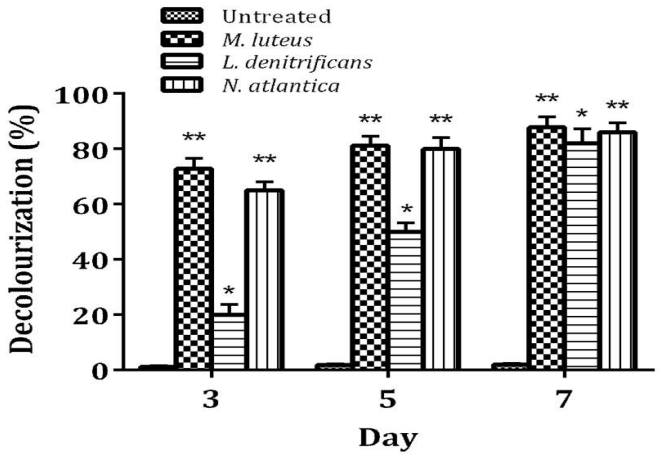

Figure-2

Decolourization of Novacron Orange W3R by bacterial isolates. The assay was performed by using isolates *M. luteus, L. denitrificans and N. atlantica* following the similar process described in Figure 1. The data is representative of three independent experiments. Here, * = P <0.05, ** = P< 0.01 and *** = P< 0.001, P = significance level.

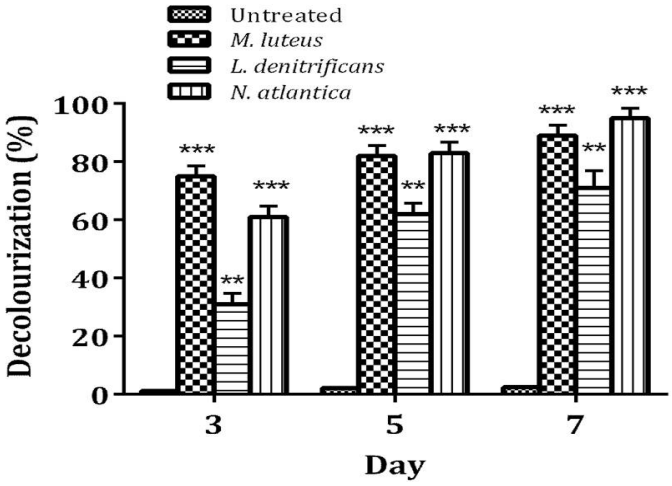

Figure-3

Decolourization of Novacron Red FNR by bacterial isolates. The assay was performed by using isolates *M. luteus, L. denitrificans and N. atlantica* following the similar process described in Figure 1. The data is representative of three independent experiments. Here, * = P <0.05, ** = P< 0.01 and *** = P< 0.001, P = significance level.

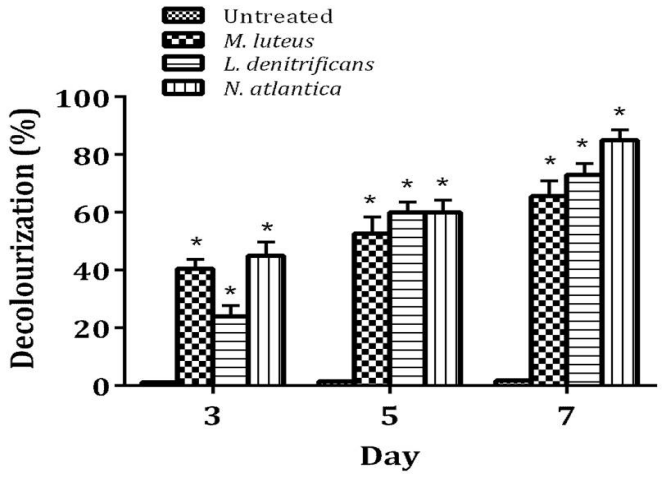

Figure-4

Decolourization of Novacron Navy WB by bacterial isolates. The assay was performed by using isolates *M. luteus, L. denitrificans and N. atlantica* following the similar process described in Figure 1. The data is representative of three independent experiments. Here, * = P <0.05, ** = P< 0.01 and *** = P< 0.001, P = significance level.

The dye supposed to be the most resistant against biodegradation is Novacron Yellow FN2R. *N. atlantica* exhibited highest 80% decolourization of this dye though it is the lowest among the highest decolourization rates achieved against each particular dye. *M. luteus* decolourized only 61% of this dye after 7 days of incubation while *L. denitrificans* decolourized only a negligible amount (10.2%) after same days of incubation (figure 5).

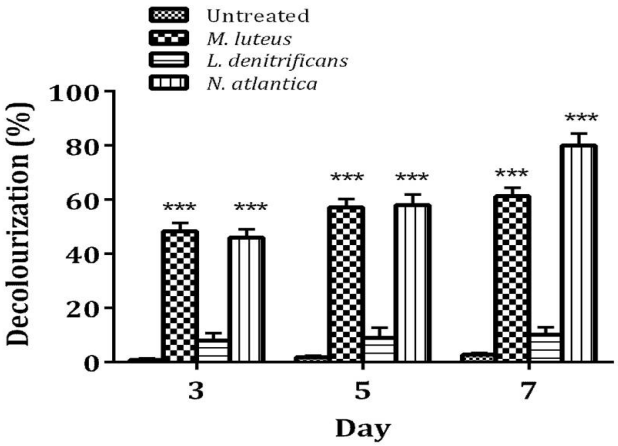

Figure-5

Decolourization of Novacron Yellow FN2R by bacterial isolates. The assay was performed by using isolates *M. luteus, L. denitrificans and N. atlantica* following the similar process described in Figure 1. The data is representative of three independent experiments. Here, * = P <0.05, ** = P< 0.01 and *** = P< 0.001, P = significance level.

The presence of *Micrococcus* sp and *Bacillus* sp in the textile effluent is regular findings and several workers[24-26] reported the role of these bacterial genera in dye degradation. But, the role of *Listeria denitrificans* and *Nocardia atlantica* in the dye degradation is not well documented.

Micrococcus luteus manifested highest decolourization (%) against Novacron Red FNR. In one previous experiment, *Micrococcus* sp. manifested highest decolourization of Reactive Yellow 42 and Reactive Red 52 within 24h under aerobic condition without forming any toxic end products[24]. In our experiment, *Micrococcus luteus* cause more than 70% decolourization within 72 hours and finally decolourized almost 90% of Blue FNR, Red FNR and Orange W3R though they are high quality Novacron dyes having impressive robustness, wet fastness and high fixation capability. Thus, revealing *M. luteus* as one of the few prominent Novacrone dye degrader.

Listeria denitrificans is also a potential Novacorn dye degrader found to act best against Blue FNR and Orange W3R (more than 80% decolourization), moderately against Red FNR and Navy WB (more than 70% decolourization). However, it didn't cause any notable decolourization of Yellow FN2R.

Nocardia atlantica have been found to decolourize more than 80% of all tested dyes while causing almost complete decolourization of Blue FNR (98%) and Red FNR (95%) within 7 days of incubation. This was similar to one previous finding where they reported a maximum of 95% decolourization by a *Bacillus* sp[27]. In another experiment done for the evaluation of interactive effects of temperature, pH and enzyme concentration by response surface methodology maximum colour removal was found 98.9%; almost the same as *Nocardia atlantica* against Blue FNR in our experiment[28]. Except Novacron Orange W3R, it was the best degrader of all other dyes used in this experiment validating *Nocardia atlantica* as the most potential textile dye degrader. However, *M. luteus* and *L. denitrificans* are also potential.

Conclusion

All of the above discussion suggests that *Micrococcus luteus, Listeria denitrificans* and *Nocardia atlantica* can flourish in the toxic dye environment by utilizing them as their source of energy when other sources are limited or unavailable. All of the isolates in this study have impressive dye degrading capability and *Nocardia atlantica* being the best of all. Thus, they can be used as excellent bioagents for the bioremediation of toxic Novacron textile dyes which have been proved to be the smartest way to wrestle with dye effluent related pollution. There are a few data suggesting the dye degrading capability of *Nocardia atlantica* and *Listeria denitrificans.* Further molecular study on their enzymatic property and degradation process could reveal them as an important textile dye degrader.

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