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Study of Effect of Physiochemical Parameters on the Reproducibility (validation) of Biodegradation of Textile Dyes

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Abstract

Background: Environmental pollution due to different industrial waste has become a major problem in modern world because of their high toxic in nature. Textile wastes are carcinogenic and mutagenic contain varieties of toxic dyes and fixers. Azo dyes widely used in textile, paper and printing industries are not easily degradable and having carcinogenic nature. Due to health hazards of textile wastes, several chemical and biological methods are being used to make the discharged wastewater less toxic by degrading colors and other harmful chemicals. Present research study was aimed to evaluate the sensitivity of biodegradation process in form of Chemical oxygen demand (COD) and, to check the reproducibility of biodegradation results for validation purpose.

Method: Initially selective bacterial strains were isolated from different waste samples. COD of the three samples of dyes were performed by titration method to observe the pattern of reproducibility of the biodegradability (COD) results.

Results: Textile industry sludge wastewater samples contained different characteristics of dye degrading consortium of bacterial strains as compared to soil extracts samples whereas did not get any bacterial growth in domestic wastewater samples. It was interesting to observe that the reproducibility of COD results was very hard to get due to sensitivity of the test parameter to the prevailing environmental conditions.

Conclusion: Present study is significant for understanding the sensitivity of reproducibility or validation of biodegradation study. On the basis of present findings of experiments, we can say that only the method of determining COD could be standardized which will ensure the credibility of the results and the changed environmental temperature could affect the controlled experimental results. Present finding would give benefit to the new scientists and students for understanding the normal behavior of biodegradation study.

Introduction

Dyes are natural or synthetic compounds that can impart colors to other substances such as textile fibers [1]. Extensively, synthetic dyes are being used in paper, textile, and leather industries. They are also being applied in color photography and as an additive in petroleum products. Synthetic dyes are more superior as compared to natural dyes due to their multiple varieties of color, stability to light, temperature, detergents and microbial attacks and cost effectiveness in firmness [2, 3].

Textile and other industries produce effluents that are resistant to biological treatments due to which these wastes have become a major environmental issue. Among all industrial waste waters, textile waste is the most polluting in nature due to the presence of organic and inorganic substances along with polymers and other products with high COD, BOD, pH and color [4-7]. The untreated textile waste when mixed with surface water causes rapid depletion of dissolved oxygen due to their high COD and BOD values which are highly toxic to biological life. They adversely affect aquatic life along with interfering the biological processes such as high invisibility due to high color intensity in wastewater hinders the light penetration and leads to affect the photosynthetic process in aquatic plants [4, 8]. Due to the above-mentioned reasons, it is very important to treat the textile waste especially containing colorants along with other toxins. Moreover, the untreated textile industrial waste is a major source of phenol, metal dyes with carcinogenic and mutagenic aromatic amines [9, 10]. They severely affect the internal vital organs such as kidneys, liver and gastrointestinal tract [11].

It is now the need of the day to find some effective and economical treatment processes for biodegradation of textile wastes. Previous research reviewed some chemical processes such as coagulation, ozonation, precipitation, adsorption by activated charcoal, ultra and nano filtration [12]. electrochemical oxidation, electro-coagulation [13, 14] and resulted in bringing excess amount of chemical sludge which in itself is another problem for their disposal. Besides, these pollutants being costly and inefficient are sometimes also known to produce hazardous byproducts which affect the environment during the degradation process [15]. The purpose of research study is to determine the chances of getting the reproducible biodegradation results of textile dyes when samples were run under same conditions, which would be a very important point of concern for the research investigations running related biodegradation. Since, biological treatment processes are drastically affected by several physicochemical parameters, such as dissolved oxygen concentration, the level of agitation, pH, temperature, supplementation of different carbon and nitrogen sources, electron donor and redox mediator, dye structure and concentration which directly influence the bacterial decolonization and degradation performance of dye effluents.

Methods

Dye Samples:

In the present study, dyes were purchased from private small-scale industry located at Karachi, Pakistan. Reactive Blue, Red and Purple dyes were used as samples for biodegradation study.

Wastewater and soil samples:

Three different types of sources namely domestic wastewater, soil extract and textile industry wastewater along with sludge were used.

Bacterial Culture:

Domestic wastewater, soil extract and textile industry wastewater containing sludge were used for isolating the dye degrading bacteria. These samples were collected in June 2020.

Method of Enrichment of different natural sources with the selected dyes for the Isolation of Dye Degrading Bacterial Strains

a). Domestic wastewater: A total of 100 ppm of Reactive Blue, Red and Purple dyes was inoculated separately in 500ml TSB (Tryptic soya broth) medium containing 50ml of the domestic wastewater.

b). Soil Extract: A total of 100 ppm of Reactive Blue, Red and Purple dyes was inoculated separately in 500ml TSB containing 25g soil. The soil sample was collected from a textile industry for the isolation of dye degrading bacteria.

c). Textile Wastewater: A total of 100 ppm of Reactive Blue, Red and Purple dyes was inoculated separately in 500ml TSB medium containing 50ml of the textile wastewater with sludge. All the inoculated TSB containing domestic wastewater, soil extract and textile wastewater were incubated at 37˚C for 7-14 days to get the active/fresh cultures of dye degrading bacterial isolates. Isolation of bacterial strains from Inoculated Domestic Wastewater, Soil Extract and Textile Wastewater: Dilutions of the inoculated TSB up to 10^{-7} with respective dyes and domestic wastewater, soil extract and textile wastewater along with sludge were prepared separately. The dilutions were plated (of the respective samples) on the Tryptic Soya agar (TSA) medium plates containing respective dye as substrate. Inoculated plates were incubated at 37˚C for 24-72hrs.

After incubation, zones of clearance were noted to check the degradation effect of the bacterial isolates (5).

Biodegradation Study: To check the reproducibility of biodegradation results, COD (chemical oxygen demand) was calculated by titration method. Briefly, the sample oxidized by potassium dichromate in 50% H_2SO_4 solution at reflux temperature. The excess dichromate titrated with standard ferrous ammonium sulfate, using Orth phenanthroline ferrous complex as an indicator. COD was calculated in milligram per litter (23, 24). All experiments were repeated twice.

Results

Isolation of Bacterial strains from Domestic Wastewater:
There was no growth on TSA plates when inoculated with the pre-inoculated domestic wastewater samples with the respective dyes.

Isolation of Bacterial strains from Soil extract:
It was observed in the study that few dyes degrading bacterial strains were grown in the plates of reactive blue dye, but with very negligible biodegrading activity or zones of clearance. There was no growth found in other red and purple dye plates.

Isolation of Bacterial strains from Textile Industry Wastewater:
The bacterial strains were grown on the inoculated plates of all three dye samples. It indicates that textile waste containing sludge has different characteristics of dye degrading consortium of bacterial strains. Bacterial strains were isolated, purified and then saved for future studies.

Biodegradation:
The COD of the repeated experiments under the same physiochemical conditions were found different in the study. The maximum percentages of degradation of all three dye samples (in form of COD) in the first experiment were 96, 94 and 98% respectively (Figure 1, 2, 3). But the percentage biodegradation was decreased as 76, 75 and 67.56% respectively (Figure 4, 5, 6) when the same experiment was repeated and the percentage of biodegradation of all three dyes were calculated in the form of COD. It might be due to several reasons such as exhausted bacterial culture, or sudden change of any physiochemical parameter such as agitation, oxygen concentration, temperature being provided to the samples which effects the reproducibility of the biodegradation (COD) results or effected the biodegradation efficiency of the bacterial strains.

OBSERVATIONS

Key:
--- (red line): Percentage of biodegradation
--- (blue line): COD readings of dyes
--- (green line): Percentage Biodegradation of Reactive Blue/Red Dyes

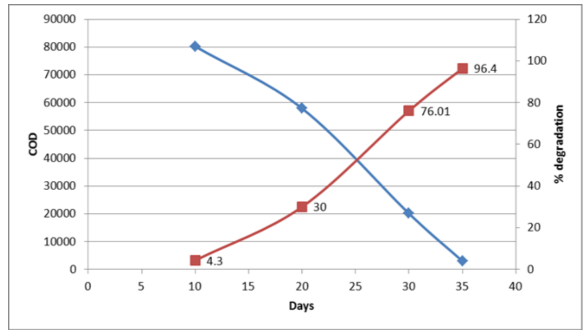

Figure 1: Percentage Biodegradation of Reactive Blue Dye

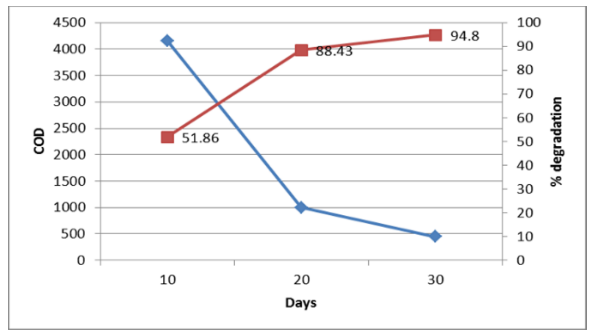

Figure 2: Percentage Biodegradation of Reactive Red Dye

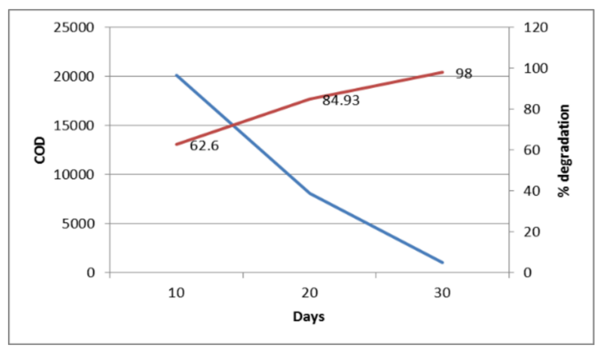

Figure 3: Percentage Biodegradation of Reactive Purple Dye

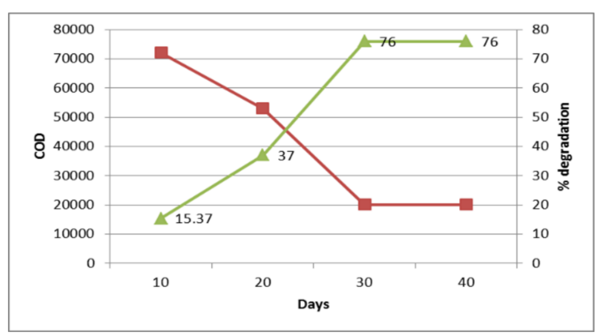

Figure 4: Percentage Biodegradation of Reactive Blue Dye

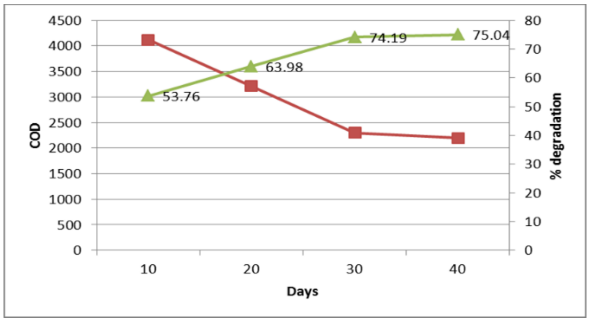

Figure 5: Percentage Biodegradation of Reactive Red Dye

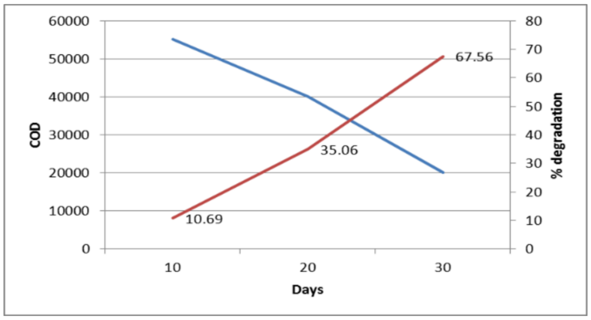

Figure 6: Percentage Biodegradation of Reactive Purple Dye

Discussion

The present study shows that textile industry sludge wastewater samples contain different characteristics of dye degrading consortium of bacterial strains than domestic wastewater and soil extracts samples. The result of the present study is agreed that sludge samples are the best sources of dye degrading bacterial strains such as *Bacillus, Alcaligenes* and *Aeromonas* species. The process of activated sludge removes approximately 90% of the inoculated dyes [16]. The activated sludge is being used to treat wastewater. It is aerated and subjected to bacterial actions, and used to remove organic matter from sewage. It is a kind of thick soft substance used for removing pollutants by biological reactions that involves oxygen from wastewater. Since biological treatments significantly depend on the presence or absence of dissolved oxygen. The soil sludge of textile industry is a very good source of active bacterial cultures which significantly degrades the inoculated dyes namely acid blue, acid red, direct yellow, direct black in 24-72 hrs under aerobic conditions [17]. It is found in the available literature that plant treatment performance of activated sludge plant was found to decrease somewhat during summer months although the provided physiochemical conditions are the same to the plant [18]. The biodegradation ability of any microbe *in vitro* conditions is not necessarily the same under *in vivo* conditions or vice versa [19].

COD is also a parameter which is considered for determining the percentage degradation of the respective dyes or any pollutants which depends upon the dissolved oxygen being provided to the bacterial/ fungal cultures to degrade the given sample. But any change in provided physiochemical conditions to the sample will directly affect the COD which determines the percentage degradation of the provided samples. For example, the maximum percentages of degradation of all three dye samples (in form of COD) in the first experiment were 96, 94 and 98% respectively (Figure 1, 2, 3). But the percentage biodegradation was decreased as 76, 75 and 67.56% respectively (Figure 4, 5, 6) when the same experiment was repeated and the percentage of biodegradation of all three dyes were calculated in the form of COD. It might be due to several reasons such as exhausted bacterial culture, or sudden change of any physicochemical parameters such as agitation, oxygen concentration, and temperature being provided to the samples which affects the reproducibility of the biodegradation (COD) results or in other words, affected the biodegradation efficiency of the bacterial strains.

Fungal dye degradation ability of fungal culture, the production of biodegrading enzymes, and their secretion are also significantly influenced by several conditions such as pH, composition of media, agitation and aeration, temperature and initial dye concentration. Even shaking of culture with the dye sample does not support decolorization [19]. The reason for least or no decolorization of the dyes at shaking condition might be, due to the competition of oxygen and dye for the reduced electron carriers under aerobic condition, although agitation supports the multiplication of bacterial culture [20]. Therefore, it could be said that even a single change in the provided conditions to the biodegradation experiment could significantly affect the biodegradation ability of the inoculated microorganism. Due to this reason, the chances of reproducibility of the same results are very low which can be observed in present study. As the percentage biodegradation is very variable or different in the repeated experiments.

The effect of different environmental factors on biodegradation process was also reported in a study. According to the author, the microbial biomass activity is one of the controlling factors for efficient biological wastewater treatment. It strongly correlated to ambient liquid temperature changes. Temperature variations not only affects the biological activities but also have a strong impact on COD, fluid viscosity, the maximum dissolved oxygen (DO) saturation levels, and on the settling velocity of biomass and ultimately affects the biodegradation process. Hence from our experimental results it was observed that that the percentage degradation (COD) of any sample can be changed in the repeated experiments due to sensitivity of the COD with different physicochemical parameters such as temperature, concentration of different chemicals in the provided medium, the production of toxic byproducts along with ever changing environmental factors. Due to which the chances of getting the reproducible results of COD are low although the biodegradation character of the bacterial consortium is still the same but affected by the surrounding experimental conditions. The studied results are also proved through different reported studies as mentioned earlier.

Competing Interests

None.

Author Contributions

Shagufta. A. Shaikh conducted the whole study from sample collection to COD analysis and continuous monitoring of samples as well as wrote and edited the manuscript. Nazia masood Ahmed retrieved, formatted the manuscript as well as analyzed the whole experimental data. Dr. Kauser Siddiqui designed the study as a Lab Incharge/ Head.

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