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Physico-Chemical and Microbiological Analysis of Textile Dyeing Effluents

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Abstract: *Textile dying industries remarkably contributes to water pollution of adjoining surface water bodies, this in turn remarkably alters biological, chemical and physical nature of the water bodies. The present study aims at studying the physico-chemical and microbiological analysis of the textile dye contaminated samples. The physiochemical analysis includes the determination of color, pH, total suspended solids (TSS), total dissolved solids (TDS), biological oxygen demand (BOD), and chemical oxygen demand (COD) using standard methods. Microbiological analysis was done by pour plate technique. Among the physico-chemical parameters, pH of the collected samples was ranged from 7.6 to 8.56 while temperature in the range of 30°C to 37°C. BOD_5, COD, TSS and TDS values were found to be 220 mg/L, 342 mg/L, 900 mg/L, 2933 mg/L for inlet and 120 mg/L, 214 mg/L, 800 mg/L, 1766 mg/L for outlet effluent, respectively. The total viable counts for all the samples were generally high and highest proportion of dye degrading microorganisms were found in the dye containing canal effluent. The study outlined the adverse effects of untreated or poorly treated effluents from dyeing industries on physicochemical and biological properties of natural water bodies. Dye degrading microorganisms, in this regard, play a significant role in the mitigation of such intensely colored dyeing effluent.*

Keywords: *Dying effluent, Dye degrading microorganisms, BOD_5, Effluent Treatment Plant, Environmental Pollution.*

I. Introduction

The world's ever increasing population and the progressive adoption of an industrial based life style has inevitably led to an increased anthropogenic impact on the biosphere. In the way of employment-intensive industrialization, textile and dying industries are playing an utmost important role in the industrial structure of Bangladesh. Accessibility of foreign markets including EU, Canada, Norway and Japan makes the industry as the leading foreign exchange earner [1].

Chittagong is the largest port city in Bangladesh, and is industrially and commercially important in southeastern Bangladesh, which consists of 510 big garment factories and 33 textile mills, though waste water treatment systems are yet to be properly established. The environmental pollution created by textile dying industries has now become a burning issue of the nation. High amount of water, used in dyeing and washing textiles are released to the surface water system, characterized by high values of temperature, pH, BOD, COD, TSS and TDS. Pollutants in wastewater such as dyes and heavy metals (Cr, Pb, Zn etc) alter the physical, chemical and biological properties of aquatic system leading to the disruption of biodiversity. Therefore, when the effluent is discharged in the surface water system, Karnafuli River gets polluted resulting in the pollution of the Halda River (as Karnafuli is connected with Halda) and the polluted river water infiltrates through earth pores and contaminates the ground water system of Chittagong ending up in having toxic pollutants in food chain [2]. To ensure the safe disposal of industrial effluents into nearby surface water resources as well as Karnafuli River, a proper analysis on management and eco-friendly treatment practices of textile and dying industries in Chittagong is essential.

A better understanding of the physico-chemical and microbiological qualities of industrial water and their receiving points are necessary so as to guide their suitability for intended purposes. This work is therefore aimed at physico-chemical and microbiological analysis of composite samples collected from dye contaminated sites of a local textile industry in order to estimate the pollution severity and relative proportion of microorganisms involved in dye degradation.

II. Materials and methods

2.1 Chemicals

All chemicals used in the study were of analytical grade with desired purity and procured from Merck, Germany and Sigma-Aldrich, USA. Nutrient Agar (Hi Media, India)) medium was used throughout the microbiological analysis. The dyes used in the study, were reactive dyes viz. Novacron Orange FN-R, Novacron Super Black G, Novacron Brilliant Blue FN-G, Bezema Yellow S8-G and Bezema Red S2-B, was generously provided by the K.D.S Textile Mills Ltd., Chittagong.

2.2 Sampling Site

The area under study was identified based on the need, diversity and extent of pollutants produced by the textile dying industry in Chittagong city. Samples were collected from Effluent Treatment Plant (ETP) of K. D. S Textile Mills Ltd., Chittagong and nearby canal carrying the effluent. Necessary permission for sample collection was taken from the authority of the factory.

2.3 Collection of Samples

Three effluent samples from ETP inlet, ETP outlet and canal effluent; one soil sample from the nearby canal, and one sediment sample from the nearby canal were collected by following standard precautions. While collecting soil, a 4 cm depth of surface soil was dug with a sterile hand-driven auger, and an approximate of 50g soil resided beneath the dug surface soil was collected into a sterile container. The time, date, location of the sample and other relevant information's of the sampling sites were recorded. After sample collection, all samples were transported as soon as possible to the laboratory of Department of Microbiology, University of Chittagong and dispensed into two portions: one portion was for physico-chemical analysis and the other portion for microbiological analysis. The sample collected for microbiological analysis was preserved in the refrigerator at 4°C before and after the microbiological analysis

2.4 Physico-Chemical Analysis of the samples

Color, texture, temperature, pH, BOD, COD, TDS, and TSS were determined for each sample following standard methods [3].

2.4.1 Determination of Temperature and pH

Temperature of each sample was determined by glass-in-mercury thermometer. Immediately after collection of the samples pH was determined with an electric pH meter (pH Hanna Instrument Ltd., UK). For soil and sediment sample, 10 gm of the sample mixed thoroughly with 20 ml of distilled water (1:2), and the pH was determined pH by meter.

2.4.2 Determination of TDS

TDS in the effluent samples were determined by filtering 100 ml sample through a Whatman No. 42 filter paper to retain fine crystalline particles. The filtrate was transferred onto the evaporating dish and placed on water bath at 100°C until all the liquid had evaporated, leaving behind on the solid remains. The dish was then placed in an oven at 100°C for 2 hrs, and then cooled in desiccators for 30 minutes. The process of drying and cooling was carried out repeatedly, in the same manner, until a constant weight was acquired. The TDS of a given sample was calculated from the following equation:

$$\text{Total Dissolved Solids (mg/L)} = \frac{(W2-W1)}{V} \times 1000 \quad \ldots\ldots (1)$$

Where,
W_1= weight of the empty dish (mg)
W_2= weight of the dish with filtrate residue after evaporation (mg)
V= volume of the sample

2.4.3 Determination of TSS

100 ml of the effluent sample was filtered through Whattman No. 42 filter paper. After filtration, the filter paper was dried at 100°C until a constant weight was achieved. The following formula was used to calculate the TSS in the effluent sample:

$$\text{Total Suspended Solids (mg/L)} = \frac{(W2-W1)}{V} \times 1000 \quad \ldots\ldots (2)$$

Where,
W_1= weight of the filter paper (mg)
W_2= weight of the filter paper and effluent residue after drying (mg)
V= volume of the sample

2.4.4 Determination of BOD

BOD of the effluent samples was determined by Winkler method. The assay was carried out by the measurement of dissolved oxygen content of the samples before and after 5 days of incubation at 20°C. The sample was freed from residual chlorine using $NaSO_4$ solution. Four times dilution of the sample were made in order to get the depletion in the range of 40% to 70%. The dilution water was prepared by aerating (bubbling compressed air) for 1-2 days to attain dissolved oxygen saturation.

The formula for calculating BOD is stated below-

BOD_5 (mg/L) = $\frac{D1-D2}{P}$……………………………………………….(3)

Where,

D_1 = DO of diluted sample immediately after preparation (mg/L)
D_2 = DO of diluted sample after 5 day of incubation at 20°C (in mg/L)
P = Decimal volumetric fraction of sample used

2.4.5 Determination of COD

COD of the effluent samples was determined by the Open Reflux method. After refluxing the sample with a known amount of standard potassium dichromate ($K_2Cr_2O_7$), the amount of dichromate consumed was found out by back titration with standard ferrous ammonium sulfate [$(Fe(NH_4)_2(SO_4)_2.6H_2O)$] (Mohr salt) and sulfuric acid (H_2SO_4), respectively in the presence of silver sulfate as catalyst. A blank was also run simultaneously.

COD was calculated using the following formula**:**

COD = $\frac{(A-B) \times N \times 1000 \times 8}{volume\ of\ the\ sample\ (mL)}$………………………………… (4)

Where,

A = amount of 0.1N Mohr salt needed in neutralizing the control mixture
B = amount of 0.1N Mohr salt needed in neutralizing the test mixture
N = normality or strength of Mohr salt.

2.5 Microbiological analysis of the collected samples

Total viable bacterial count of the samples was enumerated by serial dilution plate count method on nutrient agar medium [4]. Briefly, Serial dilution was carried out up to 10^{-6} dilutions from which an aliquot of 1 mL of each dilution was aseptically poured into duplicate sterile petriplate, and sterile melted (around 40-45°C) nutrient agar poured over it, rotated clockwise-anticlockwise, allowed to solidify, and finally, incubated at inverted position at 37°C for 48 hours. After incubation, the plates having well-spaced colonies (30-300) were selected for counting and the colonies were counted by a colony counter (Stuart Scientific, U. K.). Total viable bacterial count per mL or per gram were calculated by multiplying the average number of colonies per plate by reciprocal of the dilution and expressed as colony forming units (CFU) per mL or per gram of sample [5]. Similarly, total dye degrading bacterial count were enumerated by supplementing with 100 mg/L dye mixture (1:1 mixture of five experimental dyes viz. Novacron Orange FN-R, Novacron Super Black G, Novacron Brilliant Blue FN-G, Bezema Yellow S8-G and Bezema Red S2-B) within the nutrient agar medium.

III. Results and Discussion

3.1 Sampling

Samples were collected from different places of the K.D.S textile industries Ltd., Chittagong. Inlet effluent, outlet effluent, nearby canal sediment, soil and canal effluent contaminated with dying effluent were collected and relevant information's were recorded at the site (**Table 1**).

Table 1. Sampling and relevant information's

Sample ID.	Sample name	Collection Date	Collection Time	Place of Collection
1A	Inlet effluent (IE)	01.06.2013	9.30am	Before treatment in ETP
1B	Outlet effluent(OE)	01.06.2013	9.30am	After treatment in ETP
1C	Soil	01.06.2013	10.45am	Soil (5cm depth) from canal side
1D	Canal effluent (CE)	01.06.2013	10.50am	Nearby canal beside the mill
1E	Canal sediment (CS)	01.06.2013	11.00am	Nearby canal beside the mill

3.2 Physico-chemical analysis of collected samples

Physico-chemical parameters such as color, temperature and pH of the sample were recorded during sampling on the site and samples were brought to the laboratory with maintained temperature below 4°C and processed as early as possible. BOD, COD, TSS and TDS were measured on the sampling day as per as **Table 2**. The level of pollution of the effluents was determined by comparing the observed values of the various parameters (color, temperature, pH, BOD, COD, TDS and TSS) with the inland surface water standard values recommended by Department of Environment [6], Bangladesh. However, no vulnerable impacts of water quality for K.D.S Textile mills Ltd, Chittagong, except TSS value (which was higher than the DoE specification) was found because of using their ETP properly. The overall physicochemical parameters such as TSS, TDS, BOD and COD of the effluent samples showed deviation from DoE specification when treated effluents get mixed with nearby canal water.

Color is contributed to a water body by the dissolved compounds (dyes and pigments). The effluent color was black due to mixture of various dyes and chemicals used in the dyeing process as described by Buckley [7].

The temperature of the effluents was found to be similar with the temperature of another textile effluent study [8]. The impacts of temperature on diffusivities both in the air and water could influence emissions of both ammonia and sulfide in the effluents while volatilization of oil and grease that could be induced by the same high temperature could introduce organic compounds into the environment thus polluting the air [9].

The pH of the collected samples was found to be highly alkaline which was due to excessive use of carbonate, bicarbonate, H_2O_2 and NaOH during bleaching process [10].

TDS values of effluent sample were found to be compatible with the permissible limits but TSS values were found to be higher. A high content of dissolved solid elements affects the density of water, reduces the light penetration thereby decreased the photosynthesis, reduces solubility of gases (like oxygen) which results in decreased purification of wastewater by microorganisms [11].

The values of BOD and COD were within the permissible limits in the present samples. BOD directly affects the amount of dissolved oxygen (DO) in rivers and streams. The consequences of high BOD are the same as those for low DO: aquatic organisms become stressed, suffocate, and die. On the other hand, increase in COD could be attributed to an increase in the addition of both organic and inorganic contaminant entering the systems from the municipal sewage treatments plants [12].

Table 2. Physico-chemical analysis of the collected samples

Parameters	DoE standard	Inlet Effluent	Outlet Effluent	Canal Water	Soil	Canal Sediment
Color	-	Blackish	Colorless	Blackish	Black	Black
Texture	-	Liquid	Liquid	Liquid	Sandy	Mud
Temperature (ºC)	-	37	35	35	30	32
pH	6.5-9	7.6	8.38	8.56	7.7	7.92
TSS (mg/L)	100	900	800	950	-	-
TDS (mg/L)	2100	2933	1766	2550	-	-
BOD_5 (mg/L)	150	220	120	240	-	-
COD (mg/L)	200	342	214	227	-	-

As per Environmental Conservation Rules 1997, dyeing industries fall under the "Red category" which clearly means that, these factories must have Effluent Treatment Plants (ETPs) that must be operating throughout the year and all the ETPs (existing and to be constructed) must meet the national water quality standards. But, in developing countries like Bangladesh, where less attention is paid to environmental protection; environmental regulations are not effectively implemented and pollution control techniques are not yet fully developed. From 3,500 textile processing units, only 900 have ETPs [13]. Amid huge public criticism against textile dyeing and processing units for polluting water-bodies and farmland by releasing toxic chemical wastes, it has become a challenge for government and private sector to work together to promote ETP installation with clear understanding the gravity of the problem and to take necessary steps by giving proper attention to all aspects. So, it is high time, not only to meet the local laws but also to meet the buyer's requirement regarding environmental compliance; government and the private sector should come into with its full glory to solve the problem mutually.

3.3 Microbiological analysis of the collected samples

Total viable bacterial count and total dye degrading bacterial count of the collected samples were analyzed during the study period and their ratio were also revealed as per as **Table 3**.

Table 3: Total viable bacterial (TVB) and total dye degrading bacterial (TDDB) count

Sample ID.	Sample Name	TVBC (cfu/mL)	DDBC (cfu/mL)	(DDBC/TVBC Ratio×100)
1A	Inlet	6.8×10^5	6.9×10^4	10.14
1B	Outlet	6.5×10^5	5.4×10^4	83.07
1C	Soil	18.7×10^7	7.0×10^5	0.37
1D	Canal effluent	12.6×10^7	5.4×10^4	0.04
1E	Canal sediment	8.0×10^5	4.6×10^4	5.75

The total number of viable bacteria during the study period ranged from 6.5×10^5 to 18.7×10^7. Previous studies reported that total viable bacteria in the dye contaminated samples ranged from 6.3×10^4 to 2.8×10^8 cfu/mL [14]. Whereas the total number of dye degrading bacteria ranged from 4.6×10^4 to 7.0×10^5. The bacterial counts reflect that the textile dyeing effluent is a good source of nutrients for certain bacteria [6]. High DDB/TVB ratio were recorded is 83.07% in outlet sample, which may be associated with the slow dye

degrading activities of the dye degraders despite of the presence of dyes or may be due to the lack of sufficient nutrients in ETP treated effluent. Lowest DDB/TVB ratio were recorded is 0.04% in canal effluent sample, which may be due to the proliferation of dye degrading bacteria. A similar study was carried out to find out total hydrocarbon degrading bacteria and HDB/TVB ratio in oil contaminated surface sediment samples [15]**.**

IV. Conclusion

The textile industries, especially dye manufacturers are facing regulations and standards for their discharge effluents. The study showed a need for a continuous pollution monitoring program for the dying effluent discharged into the adjoining natural water bodies in Chittagong city. In addition to this provincial, government and private sectors should evolve measures to check and ensure that discharged effluents must comply with the specifications. Installation and operation of centralized effluent treatment plant utilizing the indigenous dye degrading microbial communities along with the physico-chemical treatment seems to be eco-friendly and cost-effective.

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