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Isolation of Mixed Bacterial Culture from Rajshahi Silk Industrial Zone and Their Efficiency in Azo Dye Decolorization

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Abstract

Background: Textile wastewater has become one of the major sources of severe environmental pollution due to the increasing demand for textile products around the world. It contains toxic azo dyes which adversely affect the environment including water bodies, soil and all living organisms. Dye decolorization through microbial community is an area of extensive research; in addition to, removal of azo dyes from contaminated sites using microorganisms is an environmental-friendly and cost-competitive alternative to chemical decomposition processes. Hence, this study aimed to isolate some local bacterial strains, which possessed the ability to decolorize azo dye containing wastewater. **Methods:** The strains were isolated as mixed bacterial culture from textile effluent collected from Rajshahi silk industrial zone, Bangladesh. Different experiments were set up including-isolation of mixed bacterial culture, growth characteristics observation and optimization, finally treatment of dye containing wastewater. **Results:** The optimum growth of this culture was observed in LB medium containing 10g/L peptone and 5 g/L yeast extract, and the temperature ranges from 30 to 40°C, pH 7.2 to 7.5 and in the presence of light. Five different (0.5%, 1%, 5%, 10%, 15%) concentrations of dye containing wastewater (ash and green color) was treated in the temperature of 37°C in anaerobic condition and in the incubation period of 0 to 15days. The mixed consortia were more effective on low concentration of dye. **Conclusion:** The obtained results showed that the selected mixed culture had the ability to cleave azo bonds and thus they are capable to neutralize the textile azo dyes. It indicated that this mixed bacterial culture was able to utilize the dyes as their carbon and energy source. Extracellular enzyme of these bacteria which called azoreductase was responsible to cleave the azo bonds of used textile dyes.

Keywords: Azo Dyes, Azoreductsae, Dicolorization, Isolation, Mixed Bacterial Culture Textile Effluent

1. Introduction

Due to rapid industrialization South Asian countries are facing severe environmental problems. This is a very common feature where the number of polluting industries likes textile, cosmetic, paper, pharmaceutical, leather etc. is increasing day by day[1,2].

The textile industry is one of them which use synthetic dyes widely. Most of them especially azo dye are resistant to breakdown. In Bangladesh, the textile mills daily discharge huge amount of untreated effluents in the form of wastewater into public drains which at last empty into rivers. Removal of the polluting azo dyes is a serious problem, particularly for small scale

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textile industries in Bangladesh, where working and low economic conditions do not allow them to treat the wastewater before dispose into the main stream of water sources.

Wide number of industries uses azo dyes. Among the all synthetic dyes, approximately 60 to 70% azo dyes produced in the world[3]. Textile industries use these dyes widely for their bright color. Beside this, the application techniques of these kinds of dyes are very simple with low energy consumption[4]. Azo dyes contain one or more azo groups – N = N – and these groups are responsible for dye color. Due to the breakage of the bond of any compound can lose its color. However, these types of structures are not easily degradable under natural conditions and the typical conventional wastewater treatment method cannot efficiently remove this from water. Azo dyes are made in such a way by which they can resist microbial and chemical attacks and can have stability in light and during washing[5]. High concentrations of azo dyes in water bodies create several serious problems. Sunlight cannot reach to the water resources due to the presence textile dyes in water and reoxygenation capacity is hampered. Textile dyes badly affect the biological and physiological activity of aquatic life[6]. These dyes have carcinogenic effect. They cause various types of tissular changes, cancers, allergies, skin irritation. There has been a link of these dyes to Bladder cancer, splenic sarcomas, hepatocarcinomas, chromosomal aberrations and nuclear anomalies in some experimental animals and in mammalian cells[7,8]. Different types of physicochemical methods for instance membrane filtration, adsorption on activated carbon, electro coagulation, flocculation, reverse osmosis, ozonation and ion exchange have been used for decolorization of dyes in wastewater. However, these methods are less efficient, costly, of limited applicability and produce wastes, which are difficult to dispose of[9]. A wide variety of microorganisms is reported to have the capability to decolorize a wide range of dyes including bacteria, fungi and algae[10]. Very often degradation of azo dyes is done by mixed culture. According to many researchers, biodegradation, detoxification and mineralization of textile dyes could be expected highly when catabolic activities within a mixed culture community complement each other. Microbial decolorization and degradation technique is an environmental-friendly and cheap in comparison to chemical decomposition processes. A wide number of researches have been performed to decolorize azo dyes under batch anaerobic condition. In comparison to other conditions, anaerobic conditions were more efficient and favorable for decolorization[11]. Setiadi and Van Loosdrecht[12] reported about 70% color removal efficiency of reactive azo dye in anaerobic reactor. In presence of microbial sludge, treatment of azo dyes in anaerobic condition can be an efficient and cheap method for removal of color from dye house effluent.

Bangladesh is heavily involved in textile production. A lot of textile mills were established in the country. Silk industrial sector is a sub-sector of textile production. Rajshahi is a well known city in northern part of Bangladesh, which is famous for its silk productin. At present, there are about 202 industries (textile, food, printing, herbal industry etc.) under the control of BSCIC in silk industrial zone, Rajshahi. These industries produce a huge amount of wastewater but there is no wastewater treatment plant in any industry. They dispose the wastewater into open environment. The city also lacks of proper sewerage system and wastewater from the factories and workshops go directly into the open drains with little or no treatment. This may have a significant effect on agricultural production where wastewater is being used. The present study deals with the isolation, purification, and screening of local bacterial isolates (from textile effluent of Rajshahi Silk Industrial area, Bangladesh) to test whether they have the capability to decolorize the azo dyes, and optimize this ability for application in textile wastewater treatment. Although research on biodegradation of azo dyes by microbial consortia has been established internationally, there is no report on decolorizing capability of locally isolated bacterial strains. Also, no investigated has been found on wastewater treatment by local bacterial strains.

2. Materials and Methods

2.1 Sampling of Textile Wastewater

Wastewater effluent from the discharge and drainage pipes of textile industries were used as the source of microorganisms. Textile wastewater sample (before dumping) were used as azo dye containing wastewater to treat. All samples were collected from Rajshahi BSCIC area, Sopura, Rajshahi. Dye concentrations of wastewater used in this study were volume based.

2.2 Aseptic Techniques

After collection of wastewater from the silk industry, it was kept at 4°C in order to prevent any activity of indigenous bacteria. Apparatus and medium were autoclaved at 121°C, 115 kPa for 20 minutes. All experiments were done inside a biosafety chamber wearing sterile disposable hand gloves. Flame sterilized loops were used for inoculations and transfers of cultures were carried out in a laminar flow cabinet to avoid contamination. The working area in the laminar flow cabinet was always sterilized by using alcohol before used. The media used in this study were Luria–Bertani (LB) medium contained 5 g/l of yeast extract, 10 g/l of peptone (20mg agar for solid medium) and Minimal-Salt (MS) medium contained 1 g/l of $(NH_4)_2SO_4$, 1.4 g/l of K_2HPO_4, 0.6 g/l of KH_2PO_4, 1 g/l of $MgSO_4$, 0.1 g/l of $CaCl_2$, 0.1 g/l of $FeSO_4$ and 10 g/l of peptone as carbon source.

2.3 Isolation of Microorganism

Firstly, 1ml textile effluent was taken by the micropipette and inoculated into the conical flask containing 100ml LB liquid medium. Then the conical flask was placed on orbital shaker for shaking 24 hours at 160 rpm. After shaking, the conical flask preserved at 4°C. It was followed by spread plate method used to isolate the mixed culture. A serial dilution was done before inoculating sample onto nutrient agar. The sample was diluted in a series of dilution tubes. 25 dilution tubes were filled with 0.9mL of distilled water respectively. Then, 0.1mL of sample was transferred into water blank followed by second transfer of 0.1mL of sample from the first dilution into another dilution tube and so on. 0.01 ml of sample from last dilution was spread over the surface of nutrient agar by using a sterilized glass spreader. All plates were incubated at 37°C for 24 hours. By observing the colony morphology, different bacterial colonies were picked up with a sterile inoculating loop and transferred into fresh LB liquid medium. The culture was maintained by proper sub-culturing process. The mixed bacterial culture was mainly consisting of some indigenous bacteria which had not been identified.

2.4 Growth Characteristics Observation

After isolation, the growth characteristics of mixed bacterial culture were observed in LB and MS media in various incubation periods, pH concentrations, carbon sources, temperatures. Effect of light was also observed. The growth of bacteria was monitored in optical density using spectrophotometer at 660nm.

2.5 Textile Wastewater Treatment

Growing cells were prepared from mixed bacterial culture in LB medium to treat the textile wastewater. The bacterial culture was ready to be used for the treatment when the optical density at 660nm reached above 1.0. The same initial cell concentrations of dye-degrading microorganisms were used to decolorize all the dyes. Decolorization in an individual dye solution could be seen visually. Two different color of dye containing wastewater sample (Acid dye ash and green) were treated. For each sample, 5 serum bottles were taken and marked (as 0.5%, 1%, 5%, 10% and 15%). Then mixed bacterial culture and wastewater sample were taken into the serum bottle for making a solution. In case of 0.5% solution (wastewater+mixed culture), 0.5ml dye containing wastewater of each color and 99.5ml mixed bacterial culture were taken. The calculation was maintained similarly in other solutions inside the serum bottles. Then the serum bottles were plugged with rubber cork, which were inserted tightly to the mouth of serum bottles. Then the serum bottles were incubated at 37°C for 15 days to observe the change of color of wastewater sample. 1ml of the culture media from different serum bottles was withdrawn at different time intervals and was centrifuged at 8000 rpm for 10 minutes to separate the bacterial cell mass. After that clear supernatant was used to measure the decolorization of dye at UV visible absorbance spectra. Controls without microorganism were always used. The percentage decolorization was calculated as follows:

$$\%\,\text{Decolorization} = \frac{\text{Initial OD} - \text{Observed OD}}{\text{Initial OD}} \times 100\%$$

3. Results and Discussion

Strong color of the textile waste water is the most serious problem of the textile effluent. Therefore, in this study, we have worked to degrade the color of textile wastewater by some local bacterial strains isolated from textile effluent drainage site. These strains were isolated by observing colony morphology (Figure 1). After isolation, the growth characteristics of mixed culture were observed both in the LB and MS (Minimal-Salt) medium. Between the two medium, the highest growth rate was observed in 72

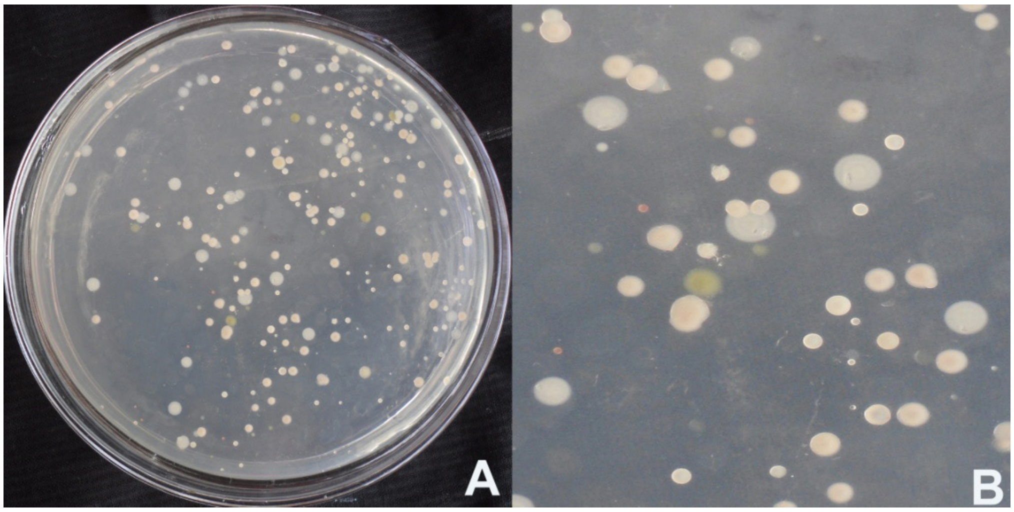


Figure 1. Figure (A and B) shows the nature of colony of the mixed bacterial culture on agar plates.

hours of incubation period in LB medium (Figure 2). Each medium were evaluated according to different pH variations ranging from7 to 7.8. The range of optimum pH variations of these strains were observed from 7.2 to 7.5 in LB medium (Figure 3). Beside this, four different types of carbon sources were used to optimize the growth rate of mixed bacterial culture. The LB media containing sugar and peptone showed the rapid and better performance on growth in comparison to MS media. Moreover, culture grown in LB medium supplemented with peptone showed the best performance (Figure 4). Light is another factor which can affect the growth rate of bacterial culture. An experiment was set up to see the effect of light on growth. Better performance of mixed bacterial culture was seen in the presence of light (Figure 5) in both medium. However, LB medium was proved better than MS medium in the growth of mixed culture under light. The experiments were run under a wide temperature ranges. Among the different temperatures (ranging from 10°C to 50°C) the maximum growths of this culture was observed from 30°C to 40°C (Figure 6). Form the above growth phenomenon, the LB medium was selected for further experiments. There are several reports on the isolation and screening of microorganisms having the capability of decolorizing different azo dyes collected from dye containing wastewater[13,14]. Only 1% microbial strains of total microbial diversity which has the capability of degradation of dye and other toxic waste materials can be cultured. Degradation of textile effluents has been carried out successfully by pure culture of bacteria and bacterial consortia.

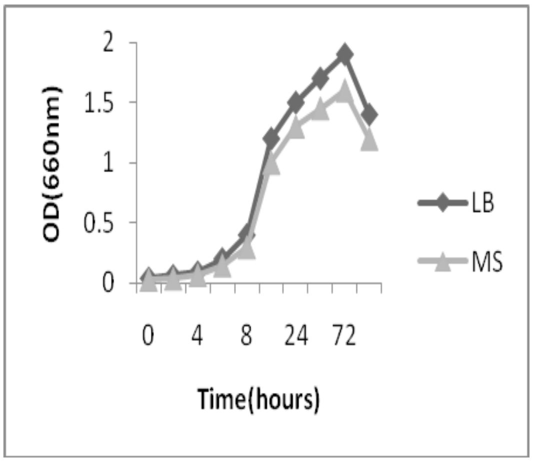


Figure 2. Effect of LB and MS media on growth rate of mixed bacterial culture in 96 hours of incubation period.

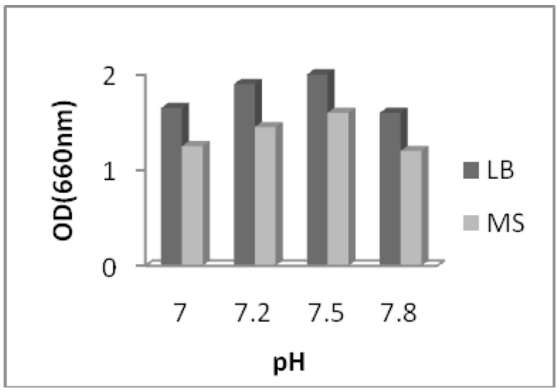


Figure 3. Effect of pH variations on the growth of mixed bacterial culture in 72 hours of incubation period.

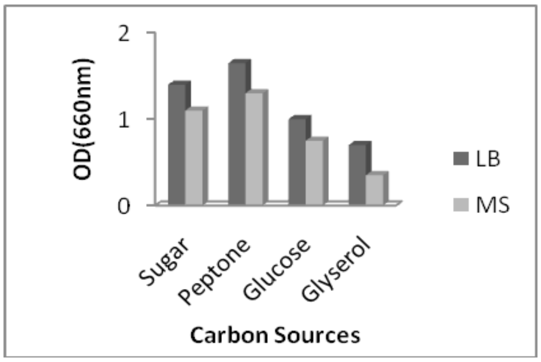


Figure 4. Effect of different types of carbon sources on the growth of mixed bacterial culture in 72 hours of incubation period.

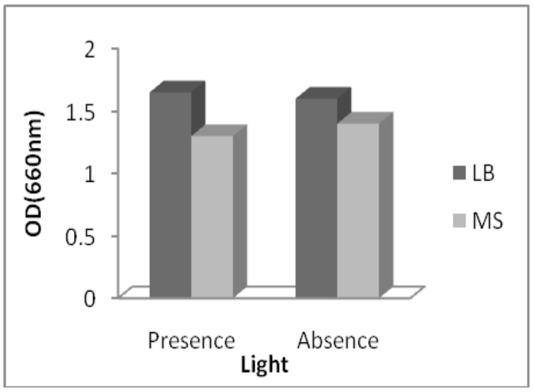


Figure 5. Growth rate of mixed bacterial in 72 hours of incubation period in presence and absence of light.

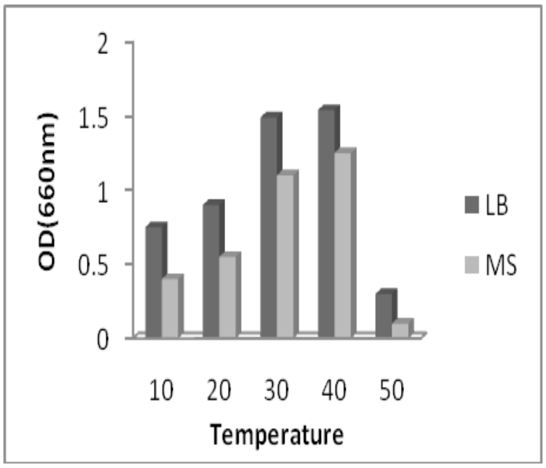
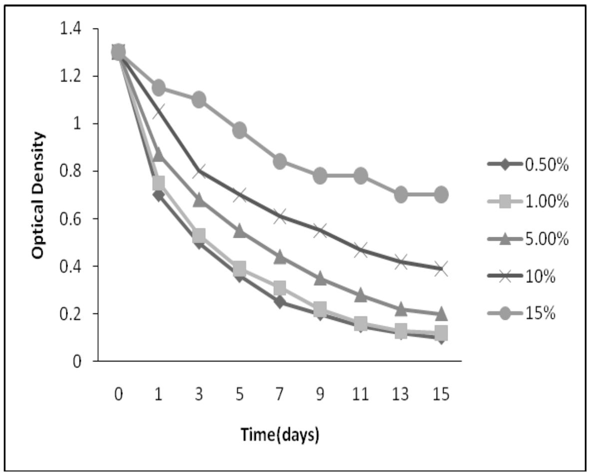
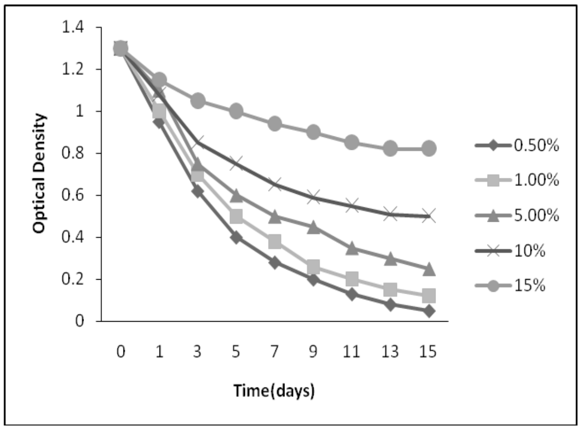


Figure 6. Growth rates of mixed bacterial culture in 72 hours of incubation period with the variations of temperature

To observe the degradation capability of mixed bacterial culture, two different colors (green and ash color) of azo dye containing wastewater were used. This experiment was conducted in the temperature of 37°C in anaerobic condition. Decolorization rate was observed in the incubation period of 0-15days. The concentrations of azo dye in wastewater sample were decreased for the time beings by the microbial attack (Figure 7) and the color was reduced gradually (Figure 8 and 9). In this investigation, it was monitored that the mixed bacterial culture was capable to decolorize azo dye containing wastewater. It indicates the breakage of nitrogen double bonds (–N=N–) of dye which is responsible for occurring pollution. Azoreductase, which thought to be the major enzyme responsible for the degradation of azo dyes in many bacterial species. Isolated bacterial strains may contain azo reductase enzyme which cleave the azo bond of (–N=N–) of those used textile dyes. Azoreductase catalyzes the azo bonds in the presence of reduction equivalent NAD(P)H, which cleaves the azo groups(-N=N-) and results azo dye degradation[15,16]. Generally thought, almost all the azo dyes have sulphonate substituent groups with high molecular weight which prevent dyes to pass through the bacterial cell membranes. Due to the impermeable nature of bacterial membranes to flavincontaining cofactors, transfer of reduction equivalents are restricted by flavins from the cytoplasm to the sulphonated azo dyes[17]. They also said this kind of mechanism is caused in bacterial cells with intact cell membranes by cytoplasmic flavin-dependent azoreductases which must be responsible for the degradation of azo dye containing sulphonated group[17].



A



B

Figure 7. Graph shows the decolorization of acid green (A) and acid ash (B) color by mixed bacterial culture in LB medium at regular time interval.

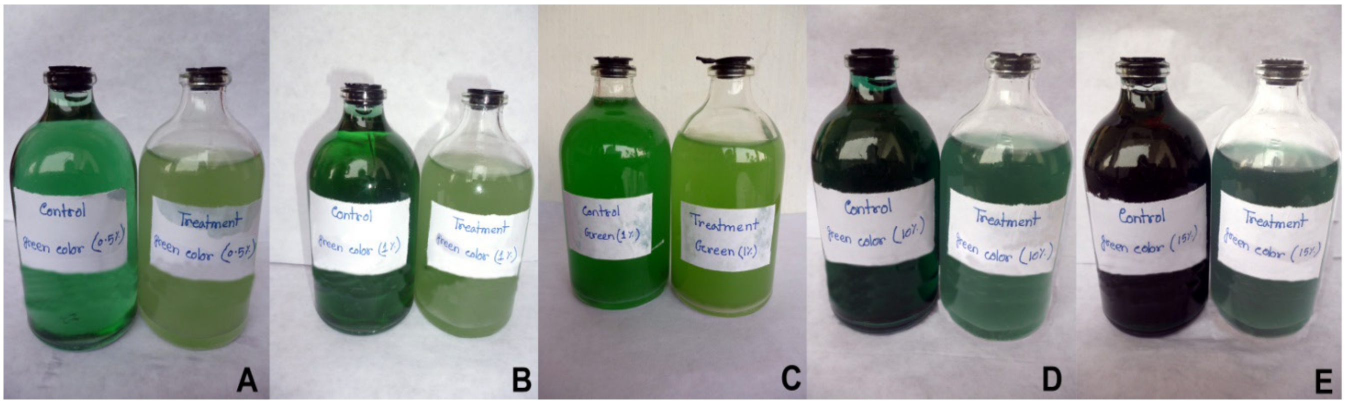


Figure 8. Effect of mixed bacterial culture on different concentrations [0.5%(A), 1%(B), 5%(C), 10%(D) and 15%(E)] of textile wastewater (acid green color).

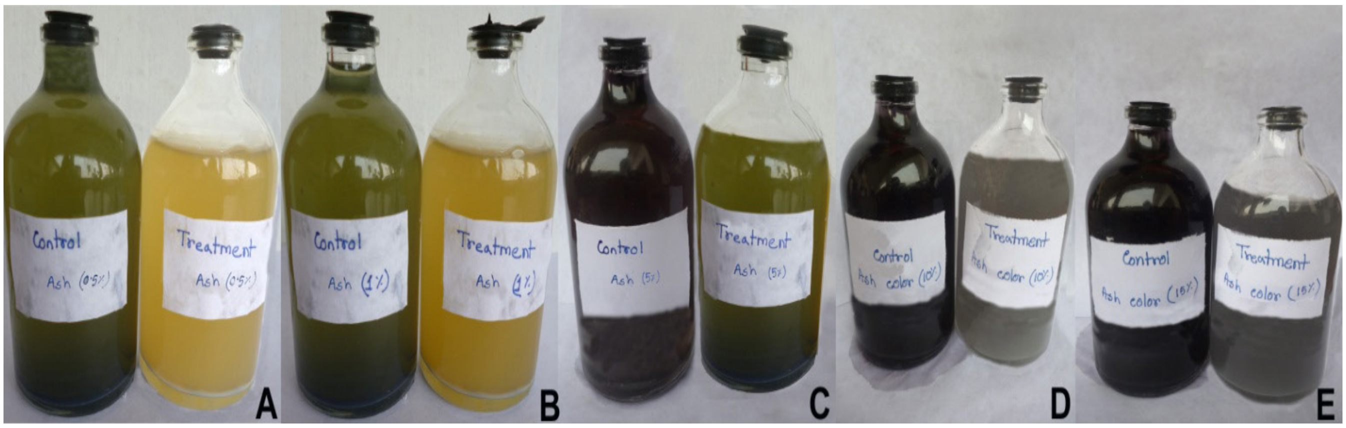
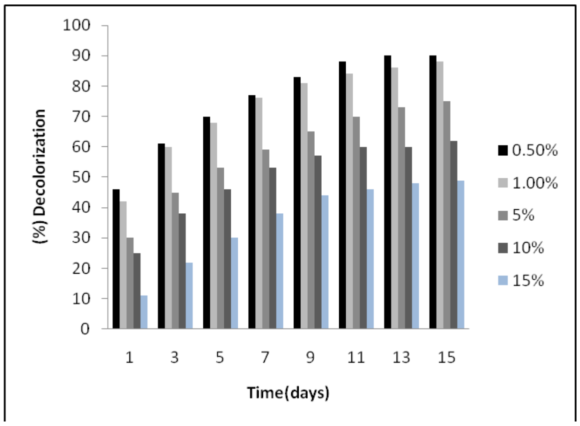
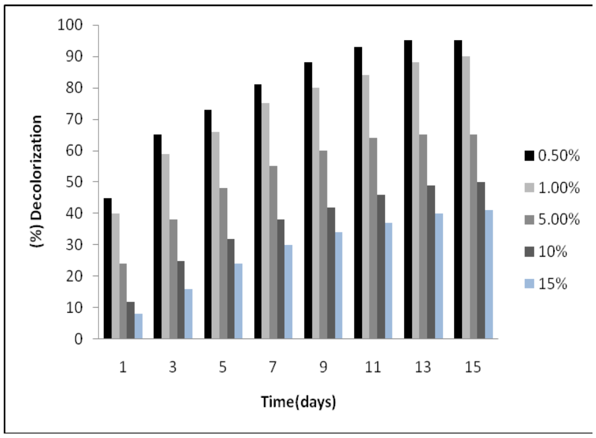


Figure 9. Effect of mixed bacterial culture on different concentrations [0.5%(A), 1%(B), 5%(C), 10%(D) and 15%(E)] of textile wastewater (acid ash color).



A



B

Figure 10. Decolorization rate acid green color (A) and acid ash color (B) at regular time interval.

Gradual reduction of color was occurred in the period of anaerobic treatment of azo dye. It was observed that mixed culture is more effective on low concentration of dye containing wastewater. About 90% decolorization was obtained from 0.5% dye containing wastewater treatment solution (Figure 10). However, when the dye concentration was high the culture showed less capability. After an initially rapid reduction of color, the rate of color removal decreased more sharply than would be expected by a first order reaction[18]. This effect was associated with the toxicity of the metabolites that were formed over the period of dye reduction. The efficiency of microbial decolorization can be influenced by dye concentration through a combination of factors including the toxicity of dye at higher concentration[19]. It takes longer time to remove the color from higher concentration of dye. When the dye concentration increased, the color removal decreased. Due to the toxicity of the dye, decolorization rates may decrease to bacterial cells for the uptake of higher concentrations of dye. Decolorization of different azo groups by isolated Exiguobacterium sp. RD3, *Pseudomonas* sp. SUK1, *Proteus vulgaris* NCIM 2027, Micrococcus glutamicus NCIM-2168 have been shown similar result[20]. Moreover, the rate of color removal can be influenced by the concentration of dye substrate. High substrate concentration can reduce decolorization of dye.

4. Conclusion

To recapitulate, it can be said that isolated mixed bacterial culture is effective to treat the dye containing wastewater. The isolation of these microorganisms from the sample gives the proof of natural adaptation of different microorganisms to survive in the presence of toxic dyes. Azo dye is highly carcinogenic compound used in textile production in Rajshahi Silk Industrial area and there is no wastewater treatment plant in this area. Therefore, it can be suggested that the textile mill authorities may build up biological, more specifically microbial wastewater treatment plant to save treatment cost and it can be environment friendly comparing with other treatment processes.

5. Acknowledgement

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