AJAB

Asian J Agri & Biol. 2018;6(4):461-471.

Original Article

Isolation, identification and characterization of dye degrading bacteria from dyeing industry effluent and degradation process optimization against Novacron Red SB

Farhana Hussain[1], Md. Reazul Karim*[1], Fahmida Hossain[2], Faisal Hasan[3]

[1]Department of Microbiology, University of Chittagong, Chittagong-4331, Bangladesh
[2]Department of Chemistry, Jahangirnagar University, Savar, Dhaka, Bangladesh
[3]University of Science and Technology, Chittagong, Chittagong-4202, Bangladesh

Received:
July 05, 2018
Accepted:
November 08, 2018
Published:
December 31, 2018

**Corresponding author email:*
reazul.m.karim@gmail.com

Abstract

Water pollution due to the desertion of enormous volume of effluent like synthetic dyes mostly azo dyes from the textile dyeing industries has become an alarming trend in the present world. Biological degradation of synthetic dye is gaining popularity because of its cost effectiveness and ecofriendly procedure. Hence, the present study was aimed to isolate and identify the indigenous azo dye decolorizing bacteria from dyeing industry effluent and to assay their dye decolorization potential in order to use them as an efficient bio-agent for decolorizing and mineralizing toxic azo dyes. Thirty indigenous bacterial colonies were isolated initially from which eight bacterial isolates were selected by further screening method, exhibiting 10% Novacron Red SB decolorization in semi-solidified screening medium. The decolorizer isolates were identified upto species as *Bacillus alvei*, *Bacillus polymyxa*, *Corynebacterium rathayi*, *Staphylococcus aureus*, *Zymomonas anaerobia*, *Bacillus megaterium*, *Aerobacter aerogenes* and *Micrococcus conglomeratus* on the basis of their morphological, cultural, physiological and biochemical characteristics. The maximum decolorization by the isolates was achieved with 5% dye concentration at 37°C temperature and pH 9 and peptone as co-substrate. So, this study demonstrates that the selected eight indigenous isolates can be used as efficient biological agent for the removal of toxic industrial novacron dyes by maintaining the above mentioned optimum value of the process parameter.

Keywords: Water pollution, Dyeing industry effluents, Azo dyes, Decolorization, Biodegradation

Introduction

Rapid industrialization has necessitated the establishment of textile industries around the world, that playing a vital role in the world economy as well as in our daily life. Water pollution, being an alarming issue caused by the production of vast amounts of waste waters containing 10-15% of dye residues from the textile industries, as for their consumption of large quantities of water (Hai et al., 2006). Textile waste water contains diverse chemical pollutants, among which azo dyes are the major classes containing carcinogenic amines, toxic heavy metals, pentachlorophenol, chlorine-bleaching, halogen carriers, free formaldehyde, biocides, fire retardants and softeners (Correia et al., 1994). Their use have

been increasing massively day by day because of their ease and cost effectiveness. Zollinger (1987) found that, approximately 100000 different dyes and pigments are used industrially and over 7×10^5 tons of these dyes are produced annually worldwide (Zollinger et al., 1987). It is estimated that 10 to 20% of the dyes used in textile processing is lost in effluent during the dyeing process (Subhathra et al. 2012). And the use of these synthetic dyes has an adverse effect on all forms of life including animals, humans and plants, because of their highly toxic and carcinogenic properties which in terms results unfit drinking water for human consumption, prevention of photosynthesis process, loss of soil productivity and hampers ecosystem integrity and plant growth (Wang et al., 2009; Pinherio et al., 2004). So there is a crying need to treat these effluents before their discharge into the environment, but treating them is very intricate. Numerous physicochemical methods were devoted for the elimination of textile effluent. Besides their efficiency, they have many limitations including expensive, labor-intensive operation and large amounts of sludge production creating secondary level of land pollution (Maier J et al. 2004 and Georgiou D et al. 2005). Being a cheaper, easier and environmentally friendlier dye removal method, bioremediation has drawn concentration as a viable alternative to physicochemical methods for the bioremediation of dyeing effluents (Walker et al., 1970; Zimmermann et al., 1984; Pasti-Grigsby et al., 1992). Bacteria have been used as an invaluable tool in effluent bioremediation because of their ubiquitous character (Olukanni et al., 2006). Usually, bacteria disintegrate azo bonds of the dyes, which result in the formation of colorless amines and subsequently simpler compounds (Stolz et al., 2001). This research study, was intended to isolate azo dye decolorizing bacteria from dyeing industry effluent and to assay their dye decolorization capability in order to use them as an efficient bioagent for decolorizing and mineralizing toxic azo dyes.

Material and Methods

Azo dye

Textile azo dye Novacron Red SB was purchased from Saad Musa Fabrics Ltd. Unit-2.Saad Musa Group, Kulgaon, Jalalabad, Chittagong, Bangladesh.

Samples collection

Textile effluent samples were collected from six different discharge points of Saad Musa Fabrics Ltd. Unit-2.Saad Musa Group, Kulgaon, Jalalabad, Chittagong, Bangladesh, in sterile plastic bottles in a cooler box and stored at 4°C.

Used culture media

Nutrient agar media (HiMedia Laboratories) was used for the enumeration and isolation of bacteria from the collected industrial effluents and also for the cultural and morphological characterization of the isolates. Semi-solidified medium which was modified version of Hayase et al. (2000) with 10% Novacron Red SB was used for the purpose of screening for dye decolorizers. The composition of the screening medium was 2.34 g K_2HPO_4, 1.33g KH_2PO_4, 0.20 g of $MgSO_4.7H_2O$, 1.00 g of $(NH_4)_2SO_4$, 0.50 g of NaCl, 0.10 g of yeast extract, 1.00 g of glucose and 1.0 ml of trace element solution per liter, adjusted to the final pH of 7.0 with 3MNaOH and HCl. The trace element solutions contained 11.90 mg l^{-1} of $CoCl_2.6H_2O$, 11.80 mg l^{-1} of $NiCl_2$, 6.30 mg l^{-1} of $CrCl_2$, 15.70 mg l^{-1} of $CuSO_4.5H_2O$, 0.97 g l^{-1} of $FeCl_3$, 0.78 g l^{-1} of $CaCl_2.2H_2O$ and 10.00mg l^{-1} of $MnCl_2.4H_2O$. Mineral salt medium (Khalid et al., 2008a) with Novacron Red SB as carbon and nitrogen source is used for the preparation of standard curve, inoculum development and in degradation process optimization assay. The composition of mineral salt medium (MSM) of pH 7.2±0.02 used for the isolation of bacteria was (g L^{-1}): NaCl (1.0), $CaCl_2\cdot2H_2O$ (0.1), $MgSO_4\cdot7H_2O$ (0.5), KH_2PO_4 (1.0) and Na_2HPO_4 (1.0), yeast extract (4.0) and agar (for solid medium only) 15.

Isolation of indigenous bacteria from dyeing effluents

Different unique bacterial colonies were isolated On the basis of their colony morphology. Then the marked observed colonies were transferred to nutrient agar slant for screening.

Screening for dye decolorizers

For extracting the dye decolorizers, the selected bacterial isolates were inoculated via stabbing into screening medium containing test tubes and observed every day in order to be decolorized from red. Isolates showing positive result in screening were selected and preserved for further analysis.

Identification of the selected isolates

The selected positive dye decolorizers were examined for their morphological properties, such as size, shape, cell arrangement and staining properties. Cultural

properties including form, color, 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, arabinose, sucrose, lactose, raffinose, inulin, starch, mannitol and glycerol were also performed. Then the isolates were selected up to species based on comparative study of the observed characteristics with the standard description of "Bergey's Manual of Determinative Bacteriology", 8th ed. (Buchanan and Gibbons, 1974).

Maximum wavelength (λ_{max}) determination of Novacron Red SB

0.015% Novacron Red SB solution was prepared with distilled water and the absorbance of the supernatants was taken in different wavelengths in spectrophotometer (Double beam spectrophotometer, Model: T 80+, Origin: UK, Source: NRS Scientific) using standard curve mode (www.optimajp.com) after centrifuging the solution at 6000 rpm for 10 minutes.

Standard curve preparation

For the preparation of standard curve 0%, 0.0003%, 0.0006%, 0.0012%, 0.0025%, 0.005%, 0.01% of dye solutions were prepared in mineral salt medium (MSM) and after centrifugation, the absorbances of Novacron Red SB were measured at 510 nm (λ_{max}). A standard curve was developed by plotting absorbance data against corresponding dye concentrations.

Inoculum development

Bacterial inoculum was prepared by incubating the MSM with eight selected isolates for 24 hours and achieving the required optical density at 625 nm wavelength.

Dye decolorization assay for degradation process optimization

The assay was performed by inoculating the prepared inoculum in conical flask containing MSM at inoculum and broth ratio of 1:50 supplemented with Novacron Red SB at varying environmental factors as dye concentrations, temperatures, pH and co-substrates in order to achieve the highest decolorization rate. During incubation, 1.5 mL aliquots were taken at defined intervals of 20, 44, 68 and 92 hours and centrifuged at 10,000 rpm for 10 minutes to measure the absorbance of the supernatants at 510 nm by using spectrophotometer (Double beam spectrophotometer, Model: T 80+, Origin: UK, Source: NRS Scientific) using ATC mode (www.optimajp.com). The experiment was conducted thrice.

Measurement of percent decolorization

From the OD values, residual dye concentration was measured from the standard curve. The rate of decolorization was calculated as

Percent of decolorization (%) = $(A_i - A_t) / A_i \times 100$, where A_i is the concentration of the initial dye solution and A_t is the concentration at cultivation time (22, 44, 68 and 92 hours).

Results

Physico-chemical characterization of the collected effluent

Immediately after the collection, the effluent samples were analyzed for various physico-chemical parameters as color, temperature, pH, BOD, COD, TDS and TSS. Total viable count was also performed of every sample. Table-1 exhibits the color, temperatures and pH of the collected effluent samples.

Isolation of indigenous bacteria

A total of 30 bacterial colonies were isolated from the collected effluent samples on basis of their colony morphology, i.e., size, shape, elevation, margin and color of the samples in order to demonstrate their decolorizing activity.

Screening for evaluating dye decolorizers

Eight bacterial isolates out of thirty indigenous isolates were preferred as Novacron Red SB decolorizers because of their significant decolorizing capability in screening medium with 10% of the dye.

Identification of the selected isolates

Identification was done on the basis of their cultural, morphological, physiological and biochemical characteristics which were introduced as *Bacillus alvei*, *Bacillus polymyxa*, *Corynebacterium rathayi*, *Staphylococcus aureus*, *Zymomonas anaerobia*, *Bacillus megaterium*, *Aerobacter aerogenes* and *Micrococcus conglomeratus.*

Preparation of standard curve

A standard curve was obtained with an equation of $y = 42.672x$ and $R^2 = 0.9984$ (Fig.1)

Peptone increase decolorization as co-substrate

The decolorization assay of Novacron Red SB was performed with three different co-substrates (ammonium acetate, glucose and peptone) at 5% initial dye concentration. No significant decolorization was observed in the presence of ammonium acetate and glucose. But, the rate of decolorization increased remarkably when peptone was used as co-substrate. When, ammonium acetate and glucose used as co substrate slight decolorization was observed after 92 hours (Fig.2 and 3). But, in case of peptone, more than 80% of the dye was decolorized within 44 hours (Fig.4).

Initial dye concentrations showed little effect on decolorization

Decolorization assay was performed in varying initial dye concentration of 5%, 10% and 15%. From the experiment, we found no significant effect of initial dye concentration on dye decolorization. In each of the varying dye concentration, almost 90% of the decolorization occurred after 92 hours of incubation. However, rapid decolorization of more than 80% occurred within 44 hours in case of 5% and 15% initial dye concentration, whereas decolorization rate increased constantly with time in 10% initial dye concentration (Fig 5, 6 and 7).

Isolates performed significant decolorization at wide range of temperatures

The decolorization assay of Novacron Red SB with time at different temperatures (25^0C, 37^0C and 45^0C) by eight isolates at 5% initial dye concentration is shown in Fig 8, 9 and 10. It is clear from the Fig 8 and 9 that at 25^0C and 37^0C decolorization rate increased with time while at 45^0C (Fig.10), some isolates failed to show significant decolorization, others are significant though.

Isolates performed notable decolorization at wide range of pH

To figure out the effect of pH on dye decolorization, three different pH (5, 7 and 9) values were set in reaction environment. In case of pH 5 and pH 7, the highest decolorization rate was achieved within 44 hour by all isolates. Whereas, in case of pH 9, though significant color removal wasn't seen after 22 hours, highest decolorization rate attained after 92 hours of incubation. (Fig 11, 12 and 13)

Table-1: Color, temperature and pH of dying effluent samples

Sample	Characteristics		
	Color	Temperature (°C)	pH
Equalization Tank	Blackish	34	9.3
Printing drain-out	Grey	26	8.8
Beaching drain-out	Turbid white	36	9.7
Washing drain-out	Blackish	35	8.4
Washing unit	Slightly turbid	26	8.3

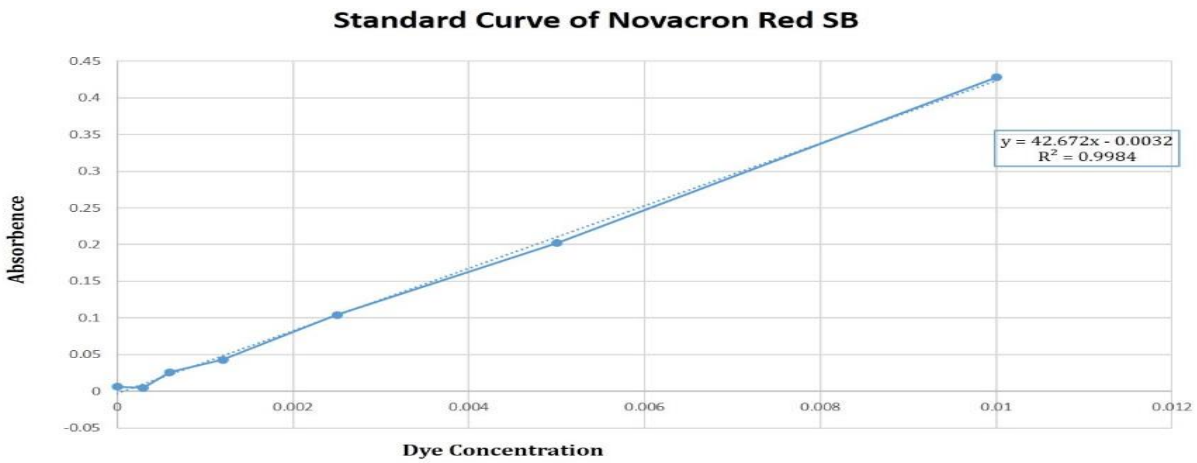

Fig.1: Standard curve of Novacron Red SB developed by preparing 0%, 0.0003%, 0.0006%, 0.0012%, 0.0025%, 0.005%, 0.01% of the dye in Mineral Salt Medium (MSM) with 0.006, 0.005, 0.026, 0.043, 0.104, 0.202, 0.428 absorbance of each of the respective dye solutions at 510 nm (λ_{max}).

Fig.2: Effect of ammonium acetate on Novacron Red SB decolorization by the selected isolates with time.

Fig.3: Effect of glucose on Novacron Red SB decolorization by the selected isolates with time.

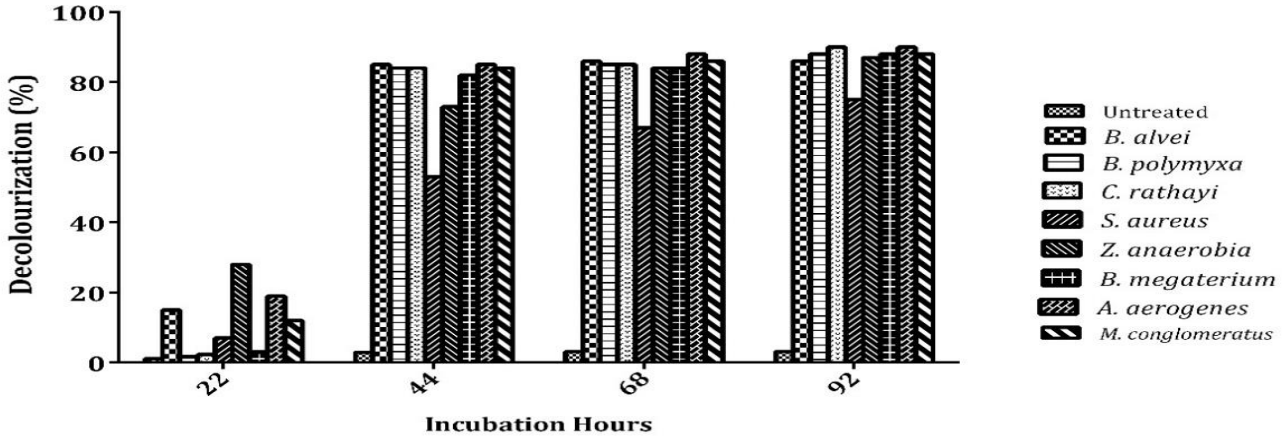

Fig.4: Effect of peptone on Novacron Red SB decolorization by the selected isolates with time.

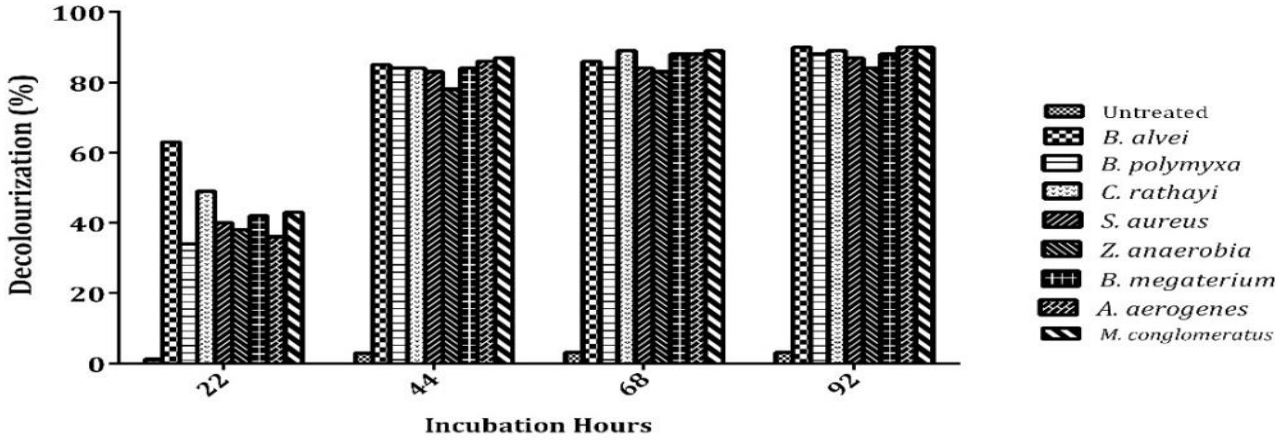

Fig.5: Effect of 5% dye conc. on Novacron Red SB decolorization by the selected isolates with time.

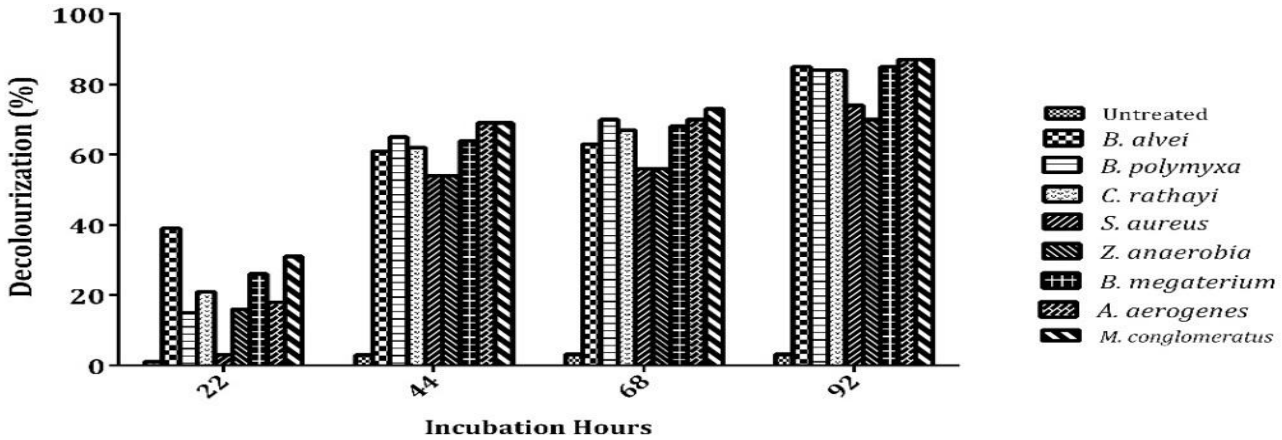

Fig.6: Effect of 10% dye conc. on Novacron Red SB decolorization by the selected isolates with time.

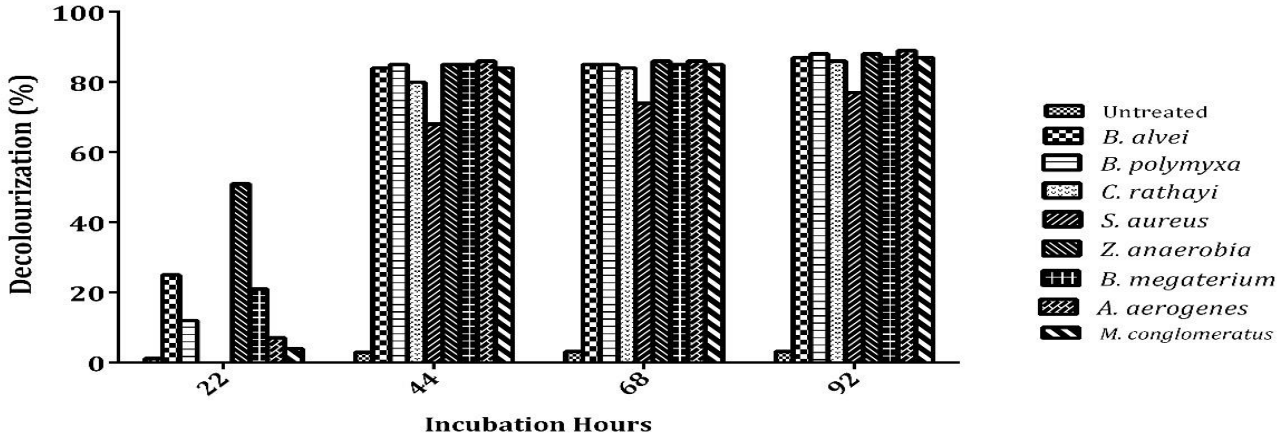

Fig.7: Effect of 15% dye conc. on Novacron Red SB decolorization by the selected isolates with time.

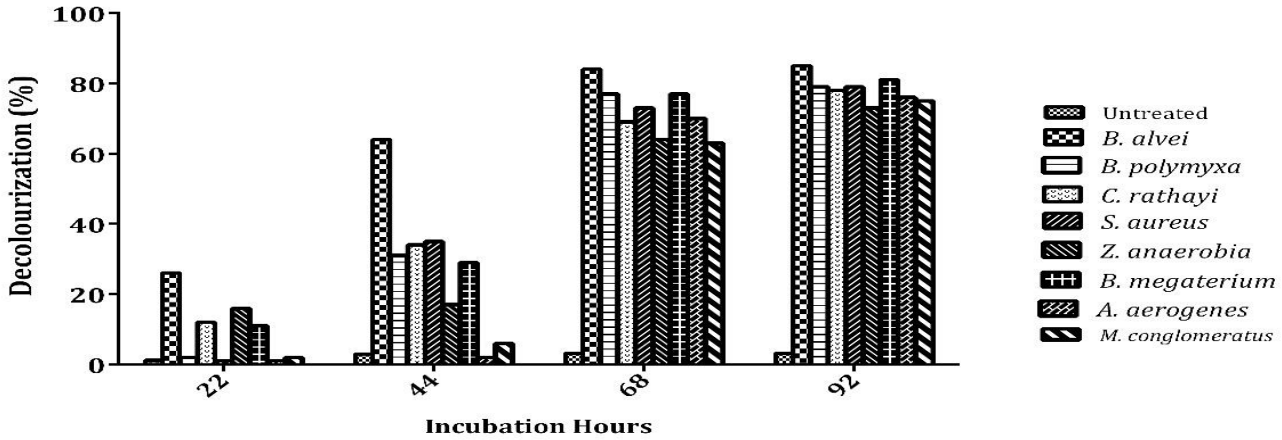

Fig.8: Effect of 25^0C temperature on Novacron Red SB decolorization by the selected isolates with time.

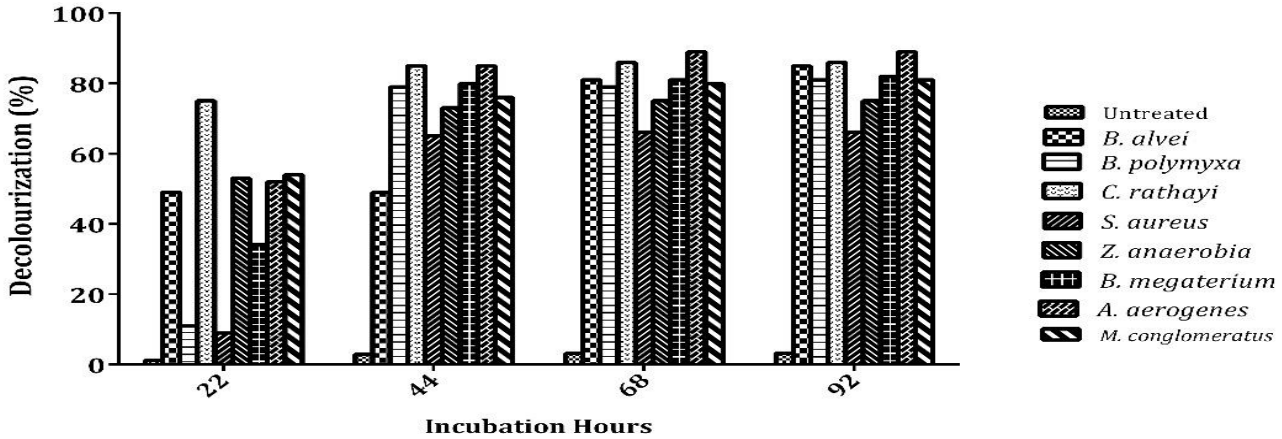

Fig.9: Effect of 37^0C temperature on Novacron Red SB decolorization by the selected isolates with time.

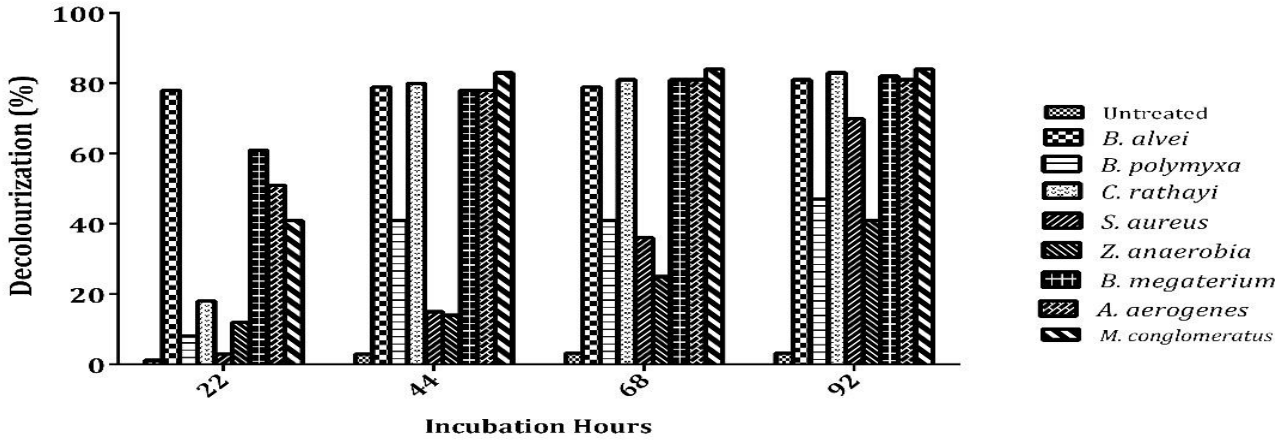

Fig.10: Effect of 45^{0}C temperature on Novacron Red SB decolorization by the selected isolates with time.

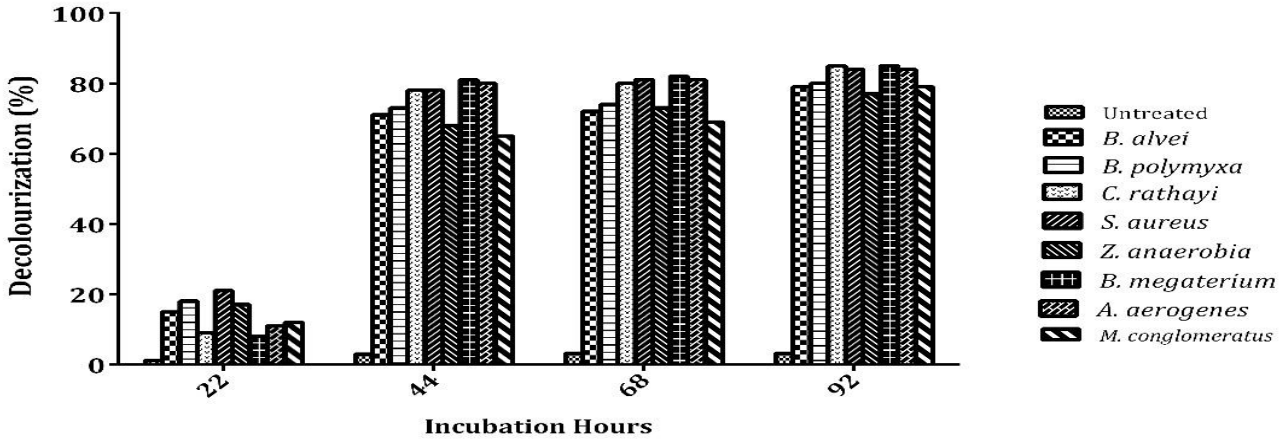

Fig.11: Effect of pH 5 on Novacron Red SB decolorization by the selected isolates with time.

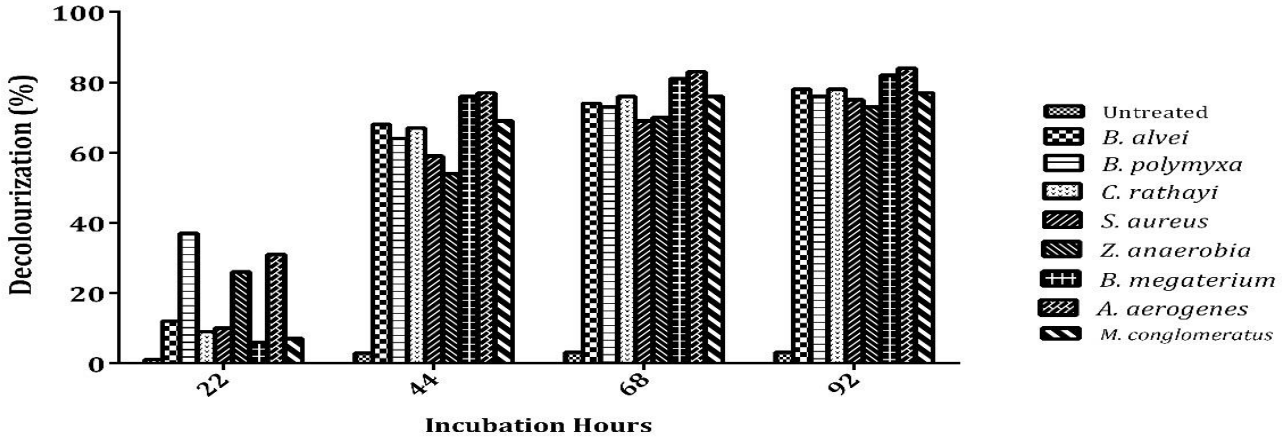

Fig.12: Effect of pH 7 on Novacron Red SB decolorization by the selected isolates with time.

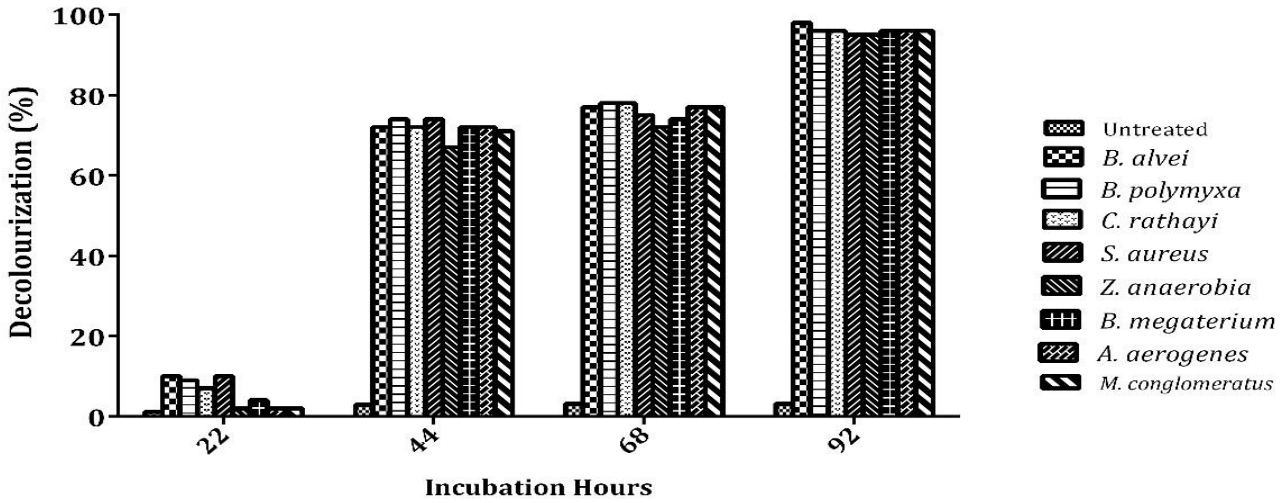

Fig.13: Effect of pH 9 on Novacron Red SB decolorization.

Discussion

In this research, textile dyeing effluents from six discharge points were collected and their physico-chemical properties were analyzed and recorded. The pH of the collected samples was in the range of 8.6 - 9.5 that was slightly alkaline and the temperature found to be in the range of 26°C - 36°C, which was found to be similar with a previous study (Mahbub et al., 2011). The color of the collected samples found by visual observation was in the range of black, due to the combination of various dyes and chemicals used in the dyeing process to turbid white. The BOD5 of the collected effluent samples were found to range from 50 – 100 mg/L and the COD values of 200-400 mg/L, which coincides with the previous study (Mahbub et al., 2011).

The isolation of the inhabiting bacteria from the collected samples was done for optimizing the environmental variables in decolorizing the studied dye in this research. From the collected sample, thirty bacterial colonies were isolated from six different effluent samples on the basis of their unique morphological and cultural characteristics. Out of these, eight isolates were selected by screening method for further decolorization study on the basis of their dye decolorizing ability on screening media containing Novacron Red SB. As these bacterial isolates are indigenous, it is clear that they use dye effluent as their energy source (Mihir et al., 2006) and thus revealed that, they can be used in degrading the textile dyes from the effluents by obtaining their nutrition.

The dye decolorization assay was performed by various parameters to assess the highest decolorization rate of the tentative dye. It was found that, among the three different co-substrates (ammonium acetate, glucose and peptone), peptone increase the decolorization rate up to 80% within 44 hours than ammonium acetate and glucose. This might be due to augmented enzyme activity by peptone as carbon source, while the others have negative impact on the enzymatic reaction. In this case, our study slightly coincides with a previous researcher according to whom complete decolorization of K-2BP was obtained in the presence of peptone or yeast extract (Guo et al., 2008).

This study also revealed that, initial dye concentration has no significant effect on dye decolorization. This means that, in case of 5% and 15% dye concentration about 80% rapid decolorization occurred within 44 hours, whereas decolorization rate increased constantly with time in 10% initial dye concentration. Temperature, being one of the most important variables in degradation process optimization was used as 25°C, 37°C and 45°C to investigate the degradation activity in present study, which explained that the decolorization increased with time at 25°C and 37°C. This result matched with a previous research,

where optimum range of temperature was reported as 28 to 35°C extract (Guo et al., 2008).

Our study also present that, at 45°C significant decolorization has rarely occurred.

Three different pH values of 5, 7 and 9 were set in the optimization assay to figure out the most favorable pH in maximum dye decolorization rate. Usually bacterial strains reveal their highest decolorization at alkaline pH and fungi at acidic pH. In our study, highest decolorization was achieved at pH 5 and 7, while no significant decolorization obtained at pH 9.

Conclusion

From the above results and discussions it can be concluded that the selected eight isolates from the textile effluent sample are efficient dye decolorizers at the optimized degradation conditions of 5% dye concentration with peptone as co-substrate at 37°C temperature and pH 7.

The properties of the isolates used in this study will be helpful not only in minimizing environmental pollution but also in providing the supply of clean safe water to the human and animals by decolorizing and mineralizing toxic azo dyes. Further molecular characterization of the isolates is needed to identify responsible genes for biodecolorization in order to achieve complete mineralization of the toxic azo dyes.

References

Buchanan RE and Gibbons NE, 1974. Bergey's Manual of Determinative Bacteriology, 8th edition. The Williams and Wilkins Company, Baltimore, USA.

Correia VM, Stephenso T and Judd SJ, 1994. Characterization of textile wastewaters. Environ. Technol. 15: 915-919.

Georgiou D, Hatiras J and Aivasidis A, 2005. Microbial immobilization in a two-stage fixed-bed-reactor pilot plant for on-site anaerobic decolorization of textile wastewater. Enzyme Microbiol. Technol. 37: 597-605.

Guo J, Zhou J, Wang D, Tian C, Wang PM and Uddin S, 2008. A novel moderately halophilic bacterium for decolorizing azo dye under high salt condition. Biodegrad. 19: 15-19.

Hai FI, Yamamoto Y and Fukushi K, 2006. Development of a submerged membrane fungi reactor for textile wastewater. Desalination. 192: 315-320.

Hayase NK, Kouno and Ushio KJ, 2000. Isolation and characterization of *Aeromonas* sp. B-5 capable of decolorizing various dyes. J. Biosci. Bioeng. 90: 570-573.

Khalid A, Arshad M and Crowley DE 2008a. Accelerated decolorization of structurally different azo dyes by newly isolated bacterial strains. Appl. Microbiol. Biotechnol. 78: 361-369.

Mahbub KR, Ferdouse J and Anwar MN, 2011. Demonstration of Decolorization of Various Dyes by Some Bacterial Isolates Recovered from Textile Effluents. Bangladesh J. Sci. Ind. Res. 46(3): 323-328.

Maier J, Kandelbauer A, Erlacher A, Cavaco Paulo A and Gubits GM, 2004. A new alkali thermostable azoreductase from *Bacillus* sp. Strain SF. Appl. Environ. Microbiol. 70: 837-844.

Mihir LS, Mahbubar RK and Farida I, 2006. Bacteria associated with textile dyeing industrial effluents and their depolarization potentiality. Bangladesh J. Microbiol. 23(1): 52-54.

Olukanni S, Osuntoki OD and Gbentle AA, 2006. Textile effluent biodegradation potentials of textile effluent adapted and non-adapted bacteria. Afr. J. Biotechnol. 20: 1980-1984.

Pasti-Grigsby MB, Paszczynski A, Goszczynski S, Crawford DL and Crawford RL, 1992. Influence of aromatic substitution patterns on azo dye degradability by *Streptomyces* sp. and *Phanerochaete chrysosporium.* Appl. Environ. Microbiol. 58(11): 3605-3613.

Pinherio HM, Touraud E and Tomas O, 2004. Aromatic amines from azo dye reduction: status review with emphasis on direct UV spectrophotometric detection in textile industry waste waters. Dyes Pigment. 61: 121-139.

Stolz A, 2001. Basic and applied aspects in the microbial degradation of azo dyes. Appl. Microbiol. Biotechnol. 56(1-2): 69-80.

Subhathra M, Prabakaran V, Kuberan T and Balamurugan I, 2012. Biodegradation of Azo dye from textile effluent by *Lysinibacillus sphaericus.* Sky J. Soil Sci. Environ. Manag. 2(1): 1-11.

Walker R, 1970. The metabolism of azo compounds: a review of the literature. Food Cosmet. Toxicol. 8: 659-676.

Wang H, Su JQ, Zheng XW, Tian Y, Xiong XJ and Zheng TL, 2009. Bacterial decolorization and degradation of the reactive dye Reactive Red 180 by *Citrobacter* sp. CK3. Int. Biodeterioration Biodegrad. 63: 395-399.

Zimmermann T, Gasser F, Kulla HG and Leisinger T, 1984. Comparisons of two bacterial azoreductases acquired during adaptation to growth on azo dyes. Arch. Microbiol. 138: 37-43.

Zollinger H, 1987. Color chemistry–syntheses, properties and applications of organic dyes and pigments. VCH, New York, USA.pp.12-13.