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Studies on Decolourization of Textile Effluent Using *Bacillus subtilis* and *Pseudomonas aeruginosa* Isolated from Textile Effluent

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Authors' contributions

This work was carried out in collaboration between all authors. Author MDM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors ODB and AB managed the analyses of the study. Author AB managed the literature searches. All authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

Aim: This research work aimed to Study the decolourization of Textile Effluent Using *Bacillus subtilis* and *Pseudomonas aeruginosa* isolated From Textile Effluent.
Study Design: This study was designed to isolate and identify *Bacillus Subtilis* and *Pseudomonas aeruginosa* from textile effluent. To use bacterial isolates in decolourization of the textile effluent individually and in the consortium (both isolates together) at three different concentrations.
Place and Duration of Study: Department of Microbiology, Nasarawa State University Keffi, Nasarawa State, Nigeria, between August 2016 and November 2017.
Methodology: Textile effluent was collected in a screw-capped sterilized bottle from the textile mill discharge point. The bacterial species of choice were isolated from the textile effluent and were subjected to bacterial decolorization individually and then in consortium using a decolorization

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medium, composed of minimal salt medium and textile effluent for a period of 12 days, the %Decolorization was measured by checking absorbance of the sample at 72hours intervals using a spectrophotometer (user 720 nm wavelength). This was repeated at different concentrations of 20 ml/250 ml, 20 ml/500 ml, and 20 ml/750 ml (v/v of textile effluent and minimal salt medium).

Results: *Bacillus subtilis* and *Pseudomonas aeruginosa* were found to have significant potential to decolourize the dye effluent. *Bacillus subtilis* produced high decolourization activity after 12 days with 58.78% at 20 ml/750 ml concentration. Whereas, *Pseudomonas aeruginosa* produced 53.46% at 20 ml/750 ml concentration after 12 days period of incubation. *Pseudomonas aeruginosa* and *Bacillus subtitles* in consortium produced the highest decolourization potential of 75.56% at concentration 20 ml/750 ml after 12 days period of incubation.

Conclusion: This study shows that *Bacillus subtilis* have comparatively higher decolourization potential than *Pseudomonas aeruginosa*. The bacteria in consortium exhibited the highest ability in the decolorizing process than the individual isolates. However, there is a need for further work to be done to validate and improve these findings.

Keywords: Biodecolorization; textile effluent; Bacillus subtilis; Pseudomonas aeruginosa; consortium.

1. INTRODUCTION

Steady growth of industries has lead to the use of diverse chemicals in our daily lives**.** Textile industry form part of the industries that uses synthetic chemicals such as Azo dyes [1]. These dyes which are aromatic compounds are increasingly used in industries due to their simple nature and less expensive. About 5-10% of the azo dyes are discharged in environment as dyestuff waste [1]. Intensive irrigation of agricultural lands with water polluted with various industrial effluents severely affects soil fertility and plant growth [1]. Dissolved substances in industrial effluents, severely alters the chemical and biological status of the soil and water, which may affect growth and productivity of plants. This also affects the susceptibility of plants to some pathogens [1]. Effluents also alter the color and quality of water bodies when discarded into them; this in turn is hazardous to aquatic organisms [2]. Any change in water quality causes several physiological and biochemical disturbances in fishes. These chemicals in the dying effluent affect the normal life of animals [3]. Effluent released into water body hinder the penetrations of sunlight thereby prevent photosynthesis from taking place [4]. Toxic compounds from the dye effluent get into aquatic organisms, pass through the food chain and ultimately reach man and cause various physiological disorders like hypertension, sporadic fever, renal damage, cramps [5]. Different methods of waste water treatment techniques such as filtration, coagulation, activated carbon and chemical flocculation have been put forward for the removal of colored textile effluent [6]. All these methods are very costly and can result in the production of large quantity of waste water and these lead to secondary level pollution of the land .However, these treatments are not found effective against the removal of color and chemicals used in the industry. Previous studies have shown that the use of bacteria in effluent treatment is effective and promising as it could bring about complete decolorization of effluent [7]. These textile effluent already contain some microbes within, it is not only advisable but also logical to use these microorganisms in decolorization of this textile effluent [6]. Bacteria are adaptive in nature, in the absence of alternative source of carbon; they are capable of deriving their nutrients from the textile effluent. Unlike conventional processes, biological treatment of textile effluent is tolerant to changes in waste composition. This may result in a short period of inactivity but do not halt the process. Safe removal of the color dye polluting the environment is necessary. Cleaning up the environment through microorganisms (Bioremediation) has been known as less expensive and ecofriendly method for disposal of textile effluent [5]. Since textile industries produce large amounts of liquid wastes that contain organic and inorganic compounds. During the dyeing processes, not all dyes that are applied to the fabrics are fixed on them and there is always a portion of these dyes that remains unfixed to the fabrics and gets washed out [6]. This study is aimed at using *Pseudomonas aruginosa* and *Bacillus subtilis* isolated from textile to decolorize the textile effluent.

2. MATERIALS AND METHODS

2.1 Sample and Sample Collection

The untreated textile effluent was collected from Teritex (No. 2 Bompai road Kano, Kano State).

The textile effluent was collected in a screw capped sterilized bottle five(5) minutes after generation of the effluent from the textile mill discharge point before getting to the receiving water body and was brought immediately to the laboratory with ice pack in a cooler box and stored at 4℃ [7]. The physical characteristics of the textile effluent was measured such as odor, color, pH using JENWAY model 3020 pH meter, temperature, Chemical Oxygen Demand (COD) and Biochemical Oxygen Demand (BOD) to determine the amount of biologically active pollutants in the textile effluent. The results were compared to Environmental Protection Agency (EPA), and Nigerian Standard for Drinking Water Quality (NSDWQ).

2.2 Microbiological Analysis of the Textile Effluent

The microbiological analysis of the textile effluent was carried out by serially diluting the textile effluent. The diluents 10^3, $I0^4$, 10^5 and 10^6 were inoculated on nutrient agar plates for the isolation of bacteria and incubated at 37℃ for 24hours. The bacterial growth was counted in colony forming units [8].

2.3 Isolation of *Bacillus subtilis* and *Pseudomonas aeruginosa*

Bacillus subtilis and *Pseudomonas aeruginosa* were isolated from the textile effluent. Briefly, One (1 ml) of the textile effluent was serially diluted aseptically into 9 ml of distilled water in a testubes into sevenfold dilutions (10^4, $I0^5$, 10^6 and 10^7) in 9ml of distilled water and one milliliter each were introduce on sterile petri dishes and sterile Nutrient Agar was added to the petri dishes using the pour plate technique [8]. The plates were incubated for 24h at 37℃ after which the colonies were counted and recorded as colony forming units per milliliter (cfu/ml) of effluent [9].

2.4 Identification of *Bacillus subtilis* and *Pseudomonas aeruginosa*

After incubation, the bacterial colonies were observed. All colonies were isolated, sub-cultured and identified using several morphological and biochemical tests including Gram's staining, motility test, Voges-Proskauer, methyl red test, citrate utilization test, starch hydrolysis test, oxidase test, catalase, Indole, Coagulase and Ureas [9].

2.5 Sterilization of the Textile effluent

The textile effluent was sterilized after the bacteria isolates were isolated from the effluent in 1000 ml Erlenmeyer flask using the autoclave at 121℃ for 15 minutes. This is done to prevent interference of another microorganism that might be present in the effluent from interacting with the selected isolates.

2.6 Media Preparation

Minimal salt medium (MSM) prepared contained the following: $(NH_4)_2SO_4$ (500 mg/l), $CaCl_2$ (14 mg/l), $MgSO_4.7H_2O$ (120 mg/l) $FeSO_4.7H_2O$ (0.13 mg/l), $ZnSO_4.7H_2O$ (5.0 mg/l), $Na_2MoO.2H_2O$ (2.5 mg/), $Na_2HPO_4.7H_2O$ (700 mg/), KH_2PO_4 (400 mg/l), pH (7), NaCl (1 mg/l) and Glucose (10 mg/l) [8].

2.7 Decolorization Medium

After preparation of minimal salt medium, it was sterilized by autoclaving at 121℃ for 15 minutes. It was then allowed to cool until ambient temperature is achieved before inoculating with test organism. Twenty milliliters (20 ml) of the collected textile effluent was added to a separate 250 ml, 500 ml, and 750 ml minimal salt medium [9].

2.8 Decolorization by Individual Isolates

Decolourization experiment was carried out in a 50ml Erlenmeyer flask. Different concentration gradients of 250 ml, 500 ml and 750 ml of the minimal salt medium were created by dispensing equal amounts (20 ml) of textile effluent into these amounts of MSM. A volume of 20 ml was then dispensed into volume 50 ml Erlenmeyer flasks and 2 ml of 24 hr bacterial isolates were inoculated into their respective flasks [9]. A control was also prepared for each concentration to which no isolates were added. All works were done in triplicates, maintaining the highest aseptic conditions before being incubated at 37℃ for a period of 12 days [9].

2.9 Decolorization by a Consortium of Bacterial Isolates

The decolourization experiment for the two isolates in consortia was carried out in a 50 ml Erlenmeyer flask. A volume of 20 ml of the decolourization media was dispensed into 50 ml Erlenmeyer flasks and 2 ml (one-milliliter

consortia of 24hr bacterial isolates each) were inoculated into their pre-labelled respective flasks. Control was also prepared for each concentration to which no isolates were added. All experiments were done in triplicates, maintaining the highest aseptic conditions before being incubated aerobically using incubator shaker at 37℃ for a period of 12 days [9].

2.10 Decolorization Assay

During incubation, Samples were drawn at the 72 hrs interval for observation. 4 ml of the mixture was centrifuged at 5000 rpm for 15 minutes. The decolourization potential of the isolates was determined by taking the absorbance reading of the cell-free supernatant using spectrophotometer at a wavelength of 720 nm [10].

The extent of decolourization was expressed as percentage (%) decolourization and estimated as;

$$\% \text{ Decolorization} = \frac{Ao - At}{Ao} \times 100\%$$

Where A_o is the initial absorbance of the textile effluent in ml/l
A_t is the final absorbance of the textile effluent in ml/l respectively [10].

3. RESULTS AND DISCUSSION

Microbial consortium showed maximum decolourization within 48 hours compared to the individual isolates (Fig.1-Fig. 3). The highest decolorization percentage for the isolates in consortia showed 63.19% at 8.0%, 64.41% at 4.0% and 75.56% at 20 ml\750 ml concentration of the effluent after day 12 period of incubation. This showed that the longer the incubation period the better decolorization process and the lower the concentration of the effluent the faster the decolorization activities of the bacterial isolates, this implies that the lower the concentration of the effluent the lesser the toxicity that will be present in the medium which will affect the decolorization potential of the bacterial isolates. This result is in agreement with the study carried out by [7,8] on the Biodegradation of dyes using a consortium of bacterial strains isolated from textile effluent. A higher degree of decolourization and mineralization can be expected when co metabolic activity within a microbial community complement each other. One organism may be able to cause a biotransformation of the dye which consequently renders it more accessible to another organism that otherwise is unable to attack the dye [12]. Thus microbial of the isolates may have an efficient enzymatic system for the cleavage of the parent dye.

3.1 Microbial count of the Isolates

The microbial count of the isolates in the textile effluent sample used for the analysis was carried out. The 10^6 dilution factor was plated on Nutrient Agar in triplicates and incubated at 37℃ for 24hours. The number of colony forming units per plate was counted and the average was calculated.

Table 1. Number of colony forming units on different plates at 10^6 dilution factor

Dilution Factor	Colony forming units (CFU/ml)
10^4	$4.2 \times 10^5 \pm 3$
10^5	$3.6 \times 10^6 \pm 4$
10^6	$2.9 \times 10^7 \pm 4$
10^7	$2.5 \times 10^8 \pm 4$

3.2 Bacterial Isolates

Bacillus subtilis and *Pseudomonas aeruginosa* were isolated from the textile effluent. These isolates where characterized based on their appearance, morphological and biochemical characteristics.

Fig. 1 shows the decolourization percentage of treated textile effluent at 20 ml/250 ml by *Bacillus subtilis*, *Pseudomonas aeruginosa and* bacteria in consortium respectively. The overall highest percentage decolourization (63.19%) was observed at day 12 by bacteria in consortium followed by *Bacillus subtilis* (47.04%) at day 12 and *Pseudomonas aeruginosa* (46.54%) also at day 12. From the results, it shows that as the incubation period increased the percentage decolourization increased and decrease in the concentration of the textile effluent increases the decolourization ability.

Fig. 2 shows the percentage of treated textile effluent at 20 ml/500 ml by *Bacillus subtilis*, *Pseudomonas aeruginosa and* bacteria in consortium respectively. The overall highest percentage decolourization (64.41%) was observed at day 12 by bacteria in consortium followed by *Bacillus subtilis* (51.35%) at day 12 and *Pseudomonas aeruginosa* (48.88%) also at day 12.

Table 2. Morphological and Biochemical Characteristics of *Bacillus subtilis* and *Pseudomonas aeruginosa* Isolated from Textile Effluent

Gram reaction	Cellular arrangement	IND	CT	COU	VP	MR	MOT	OX	UR	SH	CAT	G	L	S	Organism
+	Straight Rods	-	+	-	+	-	+	-	-	+	+	+	-	+	*Bacillus subtilis*
-	Rods	-	-	-	+	+	+	+	+	-	+	+	-	-	*Pseudomonsas aeruginosa*

KEY: IND – Indole, CT-Citrate utilization, COU-Coagulase, VP- Voges-Proskauer, MR- Methyl Red, MOT- Motility, OX–Oxidase, UR–Urease, SH–StarchHydrolysisCAT-Catalase, G–Glucose, L-Lactose and S–Sucrose

Table 3. Physicochemical characteristics of the textile effluent

Characteristics	Raw effluent
Colour	Dark Blue
Odour	Rotten egg
Temperature	40℃
pH	8.9
COD	3550
BOD	1425

Key: BOD- Biochemical oxygen demand, COD- Chemical oxygen demand

Table 4. Physicochemical parameters of raw textile effluent in comparison with standard limits for EPA and NSDWQ

Parameters	Unit	Raw effluent	EPA	NSDWQ	After treatment *B. subtilis*	With *P. aeruginosa*
Temperature	^{0}C	40	40	-	Colorless	Colorless
pH	-	9.8	7.0	7.0	Odorless	Odorless
Color	-	Dark Blue	Clear	Clear	36^{0}C	36℃
Odour	-	Rotten Egg	Odourless	Odourless	Colourless	Slightly green
COD	mg/L	3,550	120	30	189	1305
BOD	mg/L	1,425	40	50	87	234

Key: EPA- Environmental protection agency, NSDWQ- Nigerian Standard for Drinking Water Quality, BOD- Biochemical oxygen demand and COD- Chemical oxygen demand

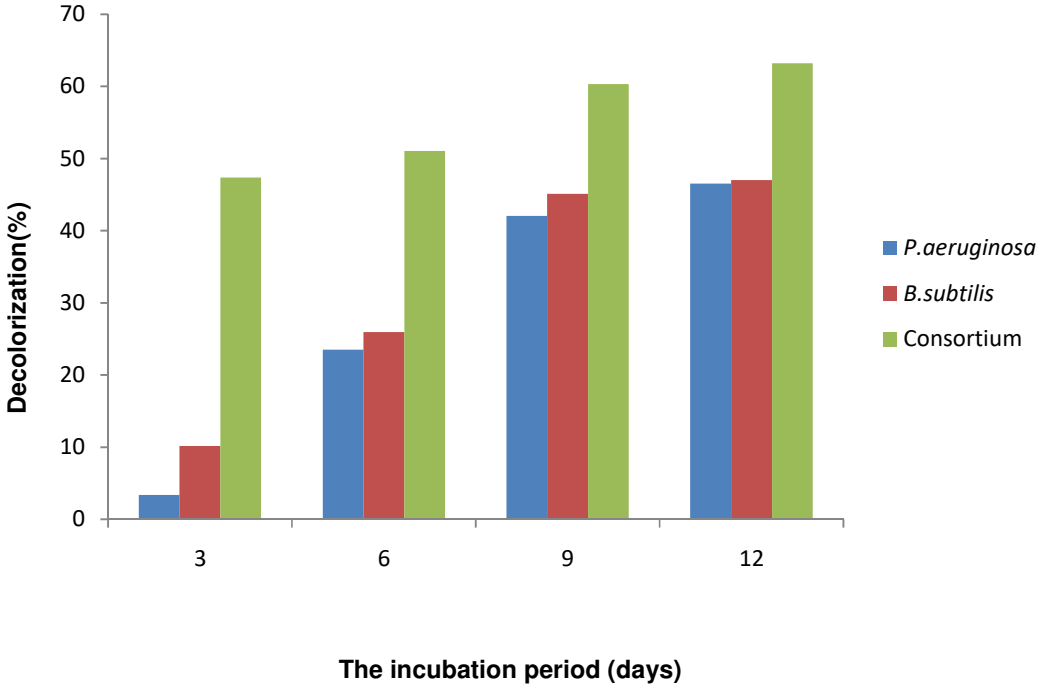

Fig. 1. Decolorization percentage of textile effluent at 20 ml/250 ml

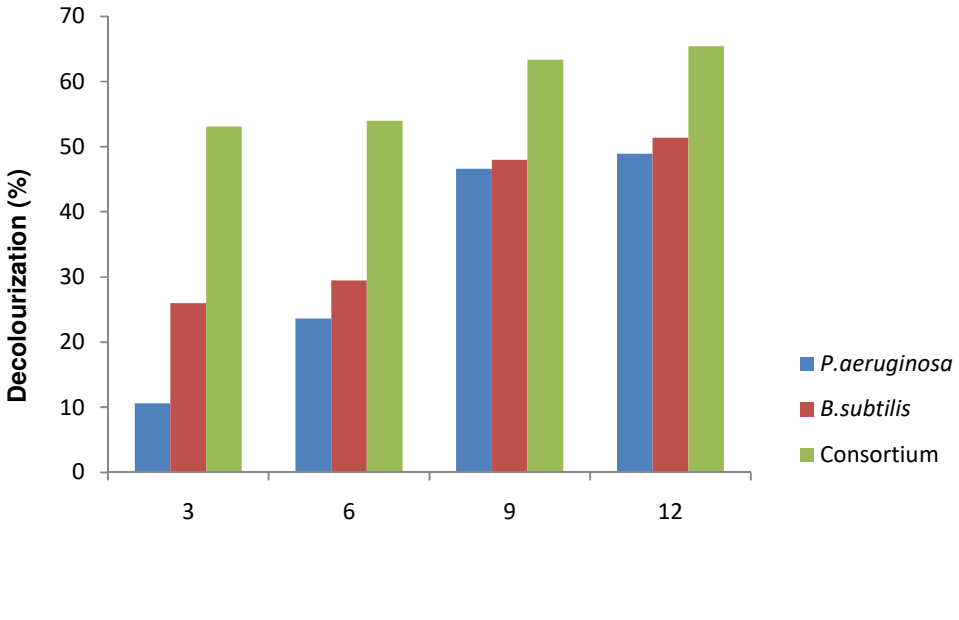

Fig. 2. Percentage decolorization of textile effluent at 20 ml/500 ml

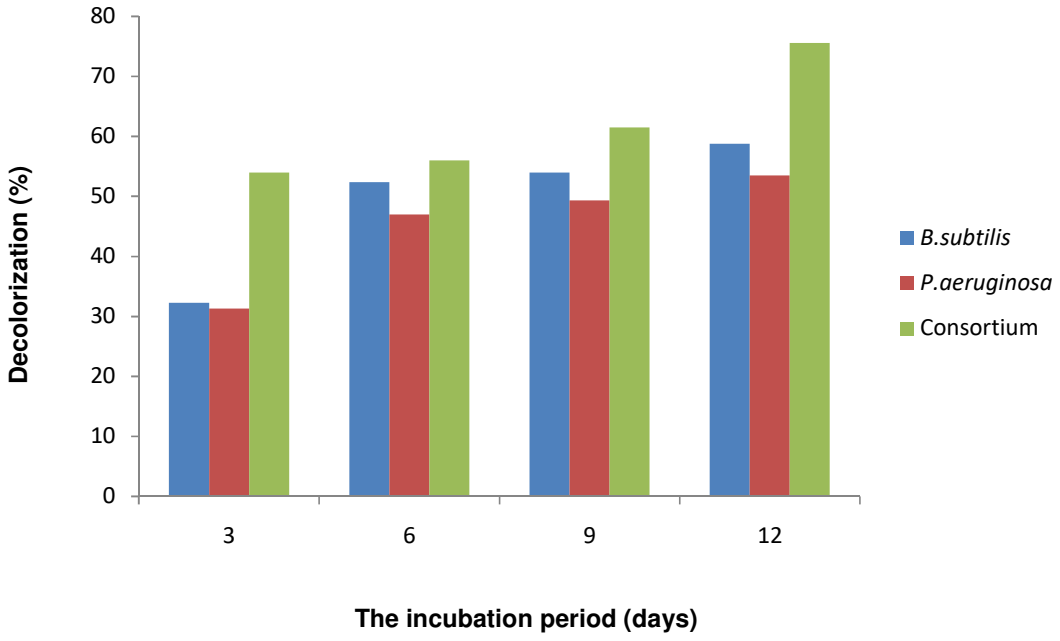

Fig. 3. Percentage decolorization of textile effluent at 20 ml/750 ml

Fig. 3 shows the percentage decolourization of treated textile effluent at 20 ml/750 ml by *Bacillus subtilis*, *Pseudomonas aeruginosa and* bacteria in consortium respectively. The overall highest percentage decolourization (75.56%) was observed at day 12 by bacteria in consortium followed by *Bacillus subtilis* (58.78%) at day 12 and *Pseudomonas aeruginosa* (53.46%) also at day 12.

4. CONCLUSION

Application of traditional waste water treatment requires enormous cost and continuous input of chemical which becomes uneconomical and causes further environmental damage. Hence economical and eco-friendly techniques using bacteria can be applied for the tuning of waste water [11,12]. Biotreatment offers easy, cheaper and effective alternative for color removal of textile dyes. Thus, by this present it was obvious that the bacterial isolates like *Bacillus subtilis* and *Pseudomonas aeruginosa* were as a good microbial source for waste water treatment specifically in biological decolorization of textile dye effluent [13,14].

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Adebiyi A, Togo C, Mutanda T, Slatter C, Whiteley CG. Decolorization and degradation of reactive blue 2 by Sulphate Reducing Bacteria (SRB) and zero valent iron in a biosulphidogenic reactor. Journal of Basic Microbiology. 2011;43:367-375.
2. Rani C, Asim K. Jana and Bansal A. Studies on biodegradation of azo dyes by white rot fungi *Daedaleq flavidain* the absence of external carbon. International Conference of Enviromental Sciences and Technology. 2011;6:147-149.
3. Robinson T, McMullan G, Marchant R, Nigam P. Remediation of dyes in textile effluent: A critical review on current treatment technologies with a proposed alternative. Bioresources Technology. 2001;77:247-255.
4. Raj A, Chandra M, Reddy H. Purohit, Copley A. Biodegradation of kraft lignin by a newly isolated bacterial strain, Aneurini *Bacillus aneurinilyticus* from the Sludge of a Pulp Paper mill. World J. Microbiolology Biotechnol. 2007;23:793-799.

5. Chen K. Decolorization of the textile dyes by newly isolated bacterial strains. Journal of Biotechnology. 2003;101:57-68.
6. Olukanni O, Osuntoki A, Gbenle G. Decolourization of azo dye by a strain of *Micrococcus* isolated from a Refuse dump soil. Biotechnology. 2009;8: 442-448.
7. Joshi PA, Sandesh J, Kirti M. Biodegredation of dyes using consortium of bacteria strain isolated from textile effluent. Journal of Experimental Biology. 2015;5(7):36 40.
8. Chang J, Lin Y. Fed-batch bioreactor strategies for microbial decorization of azodye using a *Pseudomonas luteola*strian. Biotechnology Progress. 2000;16:979-985.
9. Monica Cheesbrough. District laboratory practice in tropical countries. Part 2, Second Edition Cambridge University Press. 2010;60-80.
10. Shah MP. Biodegradation of azo dyes by three isolated bacterial strains: An environmental bioremedial approach. Journal of Microbial Biochemistry Technology. 2014;S3:007.
11. Sirinivasan V, Saravana B, Krishnakumar J. Bioremedation of textile dye effluent by *Bacillus* and *Pseudomonas* species. International Journal of Science Environment and Technology; 2014.
12. Puvaneswari N, Muthakrishnan J, Gunasekaran P. Toxicity assessment and microbial degradation of azo dyes, Department of Genetics, Centre for Execellence in Genomic Science, School of Biological Science, Madurai Kamaraji Unversity, Madurai, India Journal. 2006; 618-626.
13. McMullan G, Meehan C, Conneely A, Kirby N, Robinson T, Nigam P, Banat I, Marchant R, Symth W. Microbial decolorization and degradation of textile dyes. Applied Microbiology Biotechnology. 2001;56:81.
14. Verma P, Madamwar D. Decolorization of synthetic dyes by a newly isolated strain of *Serratia maerascens.* World Journal Biotechnology. 2003;19:615-618.

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