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MICROBIAL DEGRADATION OF SYNTHETIC THIAZOLE DYEBATH WASTEWATER

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ABSTRACT

Synthesized thiazole dye was applied on fancy gloving leather and fastness properties were determined. The dye was applied at different dyeing conditions by varying different dyeing parameters of pH, time and concentration in order to get maximum absorption and fixation. Percentage exhaustion values were observed to vary from poor to very good. Fastness properties such as light, wash, perspiration and dry/wet rub fastness were fairly good to good. The dye bath was decolorized using 1.0 ml /cfu microorganisms (Bacillus spp of bacteria and Aspergillus niger (fungi) were inoculated each into the dye bath wastewater in order to determine their decolourization potentials. The fungi, Aspergillus niger showed better decolourization of the thiazole dye bath with high degree of decolourization after 144 hours of agitation. It is therefore possible to degrade dyebath wastewater with thiazole dye effectively using Aspergillus niger at a room temperature and concentration of 1.0 ml/100 ml.

KEYWORDS: Aspergillus niger, bacillus spp, decolourization, degradation, thiazole dye

INTRODUCTION

The use of synthetic dyes has increased significantly in a number of industries, such as leather and textile dyeing or printing, cosmetics and pharmaceuticals industries. As a result, waste water from these industries has increased in the same proportion and is polluting various water bodies world-wide as 10- 15% of dyes are lost in the effluent after the dyeing process [1, 2, 3]. The leather wet finishing processes generates large amount of wastewater containing dyes as one of the main causes of water pollution. Coloured industrial effluent is the most conspicuous indicator of polluted wastewater which is aesthetically displeasing and presents adverse/negative consequences to the aquatic life [1].

Azo dyes are aromatic compounds with one or more –N=N– groups constituting the largest class of synthetic dyes used in commercial applications [4]. Most of them have been discovered to have chronic health effects in living organisms in the forms of toxicity, carcinogenicity and mutagenicity [5]. The azo bonds present in these compounds are resistant to breakdown, with the potential for persistence and accumulation in the environment [2, 6]. Several physico-chemical techniques have been proposed for treatment of colored leather processing effluents [2, 4]. These include adsorption on different materials, oxidation and precipitation by Fenton's reagent, bleaching with chloride, ozone photo degradation and membrane filtration. Others include filtration, coagulation, use of activated carbon and chemical flocculation [2,4,7]. These conventional methods are however expensive and lead to the formation of concentrated chemical sludge which is often discarded without proper treatment causing land pollution [7, 8 ,9].

For this reasons, there is a renewed focus on the microbial degradation of dyes as a more effective alternative because some microorganisms including fungi, bacteria and some algae can degrade or absorb a wide range of dyes [3,9]. It has also been discovered that bacterial degradation is normally faster compared to fungal system with regard to the decolourization and mineralization of dyes [9, 10]. It is economical, with relatively less accumulation of harmless sludge and also eco-friendly [1]. In this study, the synthesized thiazole dye was applied to fancy gloving leather and its fastness and exhaustion properties of the dye bath was evaluated. *Bacillus* spp and *Aspergillus niger*were selected and their biodegradation potential is being studied and evaluated against the thiazole dye.

MATERIALS AND METHODS

Materials and reagent

All the chemicals such as sodium bicarbonate, sodium chloride, ammonium chloride, acetic acid, urea and hydrochloric acid used were of analytical grade and were used without further purification. While conical flask, cotton wool, spectrophotometer, test tubes, syringes and other microbial materials were sterilized before each used.

Dyeing trials

The dye was used in dyeing leather at various pH, temperature and concentration. The effect of these parameters on the dye exhaustion was determined using a Jenway UV-spectrophotometer

with model no; 6305 made from Britain.The percentage exhaustion was determined by using this formula:

$$\%\ Exhaustion = \frac{ODI - OD2}{ODI} \times 100$$

Where: OD1 = optical density of dye bath before dying.

OD2 = optical density of dye bath after dyeing.

Effect of pH, temperature and concentration on dyeing

The dyeing was carried out within a temperature range of 45^{o}C - 60^{o}C at various concentrations with an interval of 0.002%-0.012% dye (w/v) dye solution. The leather samples were prepared by de-acidifying using $NaHCO_3$ to pH 5- 5.5 according to standard procedures[11]. Then the leather samples were immersed separately into the dye bath and treated with continuous agitation according to the conditions stated within a period of one hour for each sample.

FASTNESS PROPERTIES OF THE DYED LEATHER

Dry and wet rub fastness test

The leather sample was placed on the sample position and clamped firmly. The rub fastness tester (SATRA - STM 421) was switch on. The leather sample was rubbed for the stated number of revolutions (8, 32, 64 and 128). After each set of rubbing, the sample was assessed with a grey-scale. For the wet-fastness test, the felt was steamed according to standard procedure as reported in international union of physical testing procedure (IUP/401) [12]. The leather samples were then rubbed without steaming in the case of dry rub fastness test and change in coloration was assessed.

Wash fastness test

The dyed leather sample was subjected to ISO No. 3 international union of fastness testing procedure(IUF 423) colour fastness of leather to washing procedure as reported by the standard procedure of the Society of Leather Technologist and Chemist [12,13]. The leather sample with cotton was agitated in 100ml beaker containing soap solution for 30 min, as prescribed under the specified conditions. The leather and the cotton wool were then removed, rinsed and dried. The change in colour of the tested leather samples were assessed with the appropriate grey-scale.

Light fastness test

The test was carried out using standard procedure according to IUF 402[14,15]. The dyed leather sample was subjected to artificial light (xenon lamp). The grain side of the leather was exposed to light from xenon lamp under controlled conditions along with 8 blue dyed wool standards (blue scale). The light fastness was assessed by comparing the fading of the leather samples with the blue standards.

Perspiration test

The artificial perspiration mixture is defined by ISO 3160-2, IUF 426 colour fastness of leather to perspiration comprising: 5.0g/L NaCl, 5.0g/L NH_4Cl, 0.5g/L acetic acid, 0.5g/L urea and pH adjusted to 4.7 with hydrochloric acid [16]. The leather and cotton wool were immersed in an artificial perspiration solution for 30 minutes. The leather and the cotton wool were then placed between glass plates under pressure for 3hours at 37^oC and then dried. The change in colour of the dyed leather and the staining on the cotton wool was assessed with standard grey scales.

Microbial analysis.

*Aspergillus niger and Bacillus spp*were used for the study. The organisms (*Aspergillus niger*and *Bacillus spp*) were cultured on Agar slants and stored in the refrigerator at 10^oC until required for use. Chemicals used: Yeast extract, $(NH_4)_2SO_4$, KH_2PO_4, $MgSO_4$, NaCl and $CaCl_2$, according to the procedure as reported [17].

Decolourization of the dye bath wastewater

Nine different concentrations of the dye were prepared accordingly: 0.001%, 0.002%, 0.003%, 0.004%, 0.005%, 0.006%, 0.007%, 0.008%, 0.009% and sterilized with an autoclave at 121^oC. The absorbance of the samples was determined at 520nm using aJenway UV-visible spectroscopy machine model no; 6305 made from Britain. Each of the nine samples was divided into two. *Bacillus* suspension (1.0 ml /cfu) was inoculated into the first set of nine dye bath samples measuring 100ml each and the other (1.0 ml /cfu) of *Aspergillus niger* suspension was also inoculated into the remaining set as reported [17]. The samples containing *Bacillus* were incubated at 37^oC while those with *Aspergillus niger* were incubated at room temperature. The absorbance of each sample was determined at every 48 hours for 7 days and results were recorded.

Percentage decolourization of the dye bath wastewater

The absorbance taken at a fixed wavelength of 520nm was repeated every 48 hours for the 7 days. Percent decolourization was calculated using the following formula:

$$Percentage\ Decolorization\ (\%) = \frac{Initial\ Absorbance - Final\ Absorbance}{Initial\ Absorbance} \times 100$$

RESULTS AND DISCUSSION

Table1: Effect of pH on Dyeing Performance

pH	Absorb. before dyeing	Absorb. after Dyeing	Percentage exhaustion
3	0.87	0.16	81.61
4	0.86	0.19	77.91
5	0.86	0.19	77.91
6	0.86	0.19	77.91
7	0.85	0.31	63.51
8	0.86	0.38	55.81

Table 2: Effect of Temperature on Dyeing Performance

Tempt. (^{o}C)	Absorb. before dyeing	Absorb. after dyeing	Percentage exhaustion
45	0.86	0.51	33.72
50	0.86	0.49	43.02
55	0.86	0.26	69.76
60	0.85	0.32	62.35
70	0.85	0.32	62.35

Table 3: Effect of Dye Concentration on Dyeing Performance

Conc. (%)	Absorb. before dyeing	Absorb. After dyeing	Percentage exhaustion (%)
0.002	0.64	0.45	29.68
0.004	0.70	0.38	45.71
0.006	0.77	0.36	53.25
0.008	0.80	0.31	61.25
0.010	0.84	0.25	70.24
0.012	0.86	0.18	79.07

Table 4: Effect of Temperature on Fastness Properties

Temperature (^{o} C)	Light fastness	Wash fastness	Perspiration fastness
45	5	3	3
50	4	3	3
55	5	3	3
60	5	4	3
70	5	4	4

Table 5: Effect of pH on Fastness Properties

Ph	Light fastness	Wash fastness	Perspiration
3	5	3	3
4	5	3	3
5	4	3	3
6	5	4	3
7	5	4	3
8	4	4	4
9	4	4	4
10	5	4	4
11	5	4	4

Table 6: Effect of pH on Dry Rub Fastness Properties

pH	3	4	5	6	7	8	9	10	11
Number of revolutions									
8	4	4	4	4	4	4	4	4	4
16	4	¾	4	4	4	4	4	4	4
32	4	4	4	¾	4	4	4	4	4
64	4	4	4	¾	4	¾	4	4	4
128	4	4	4	4	¾	4	4	4	4

Table 7: Effect of pH on Wet Rub Fastness Properties

pH	3	4	5	6	7	8	9	10	11
Number of revolutions									
8	4	4	4	4	4	4	4	4	4
16	4	4	4	4	4	4	4	4	4
32	4	4	4	¾	4	4	4	4	4
64	4	4	4	¾	4	¾	4	4	4
128	4	4	4	4	¾	4	4	4	4

Table 8: Effect of Temperature on Dry Rub Fastness

Temperature	45	50	55	60	70
Number of revolutions					
8	4	4	4	4	4
16	4	4	4	4	4
32	4	4	4	¾	4
64	4	4	4	¾	4
128	4	4	4	4	¾

Table 9: Effect of Temperature on Wet Rub Fastness

Temperature	45	50	55	60	70
Number of revolutions					
8	4	4	¾	4	4
16	4	4	4	4	4
32	4	4	4	¾	4
64	4	4	4	¾	4
128	¾	4	4	4	¾

Table 10: Effect of Temperature on Fastness Properties

Temperature (^{o}C)	Light fastness	Wash fastness	Perspiration fastness
45	5	3	3
50	4	3	3
55	5	3	3
60	5	4	3

Table 11: Effect of Concentration on Fastness Properties

Concentration (%)	Light fastness	Wash fastness	Perspiration fastness
0.002	5	3	3
0.004	5	3	3
0.006	5	4	3
0.008	4	4	4
0.01	4	4	4
0.012	5	4	4

Table 12: Effect of Concentration on Dry Rub Fastness

Concentration (%)	0.002	0.004	0.006	0.008	0.010	0.012
Number of revolutions						
8	4	3/4	4	4	4	4
16	4	4	4	4	4	¾
32	¾	4	4	4	4	¾
64	4	4	¾	4	4	4
128	4	4	4	4	¾	4

Table 13: Effect of Concentration on Wet Rub Fastness

Concentration (%)	0.002	0.004	0.006	0.008	0.010	0.012
Number of revolutions						
8	4	4	¾	4	4	4
16	4	4	4	4	¾	4
32	¾	4	4	4	4	4
64	4	4	¾	4	4	4
128	4	4	4	4	¾	4

Results of decolonization of dyed wastewater treated with bacillus spp and aspergillus niger microorganisms is displayed in figures I and II below. The results indicate the effect of time and inoculum used for treatment of the dyed wastewater.

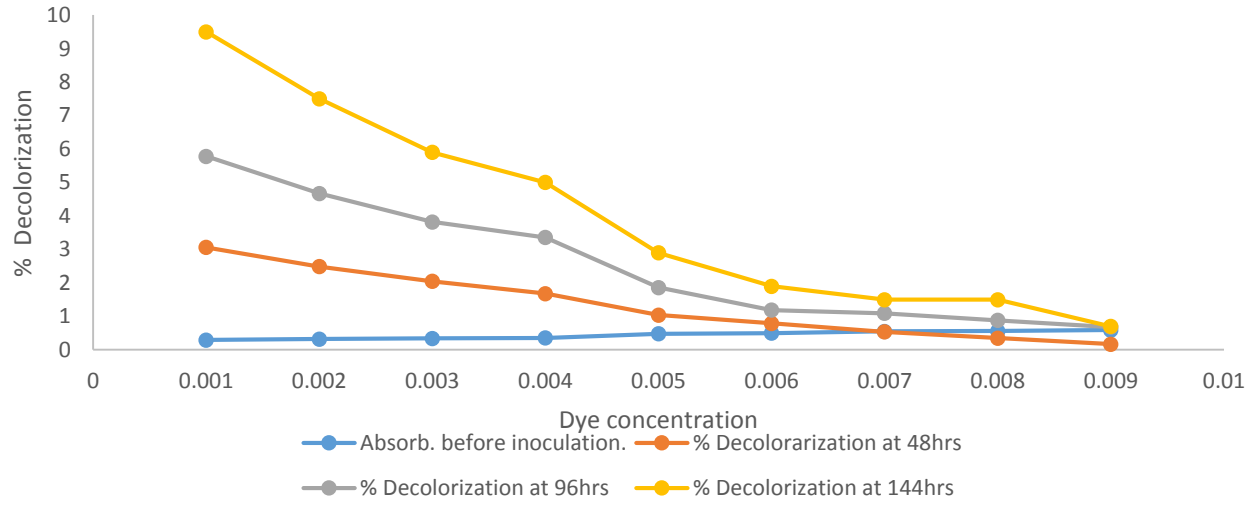

Fig 1: Decolourization of Thiazole Dye using *Bacillus*

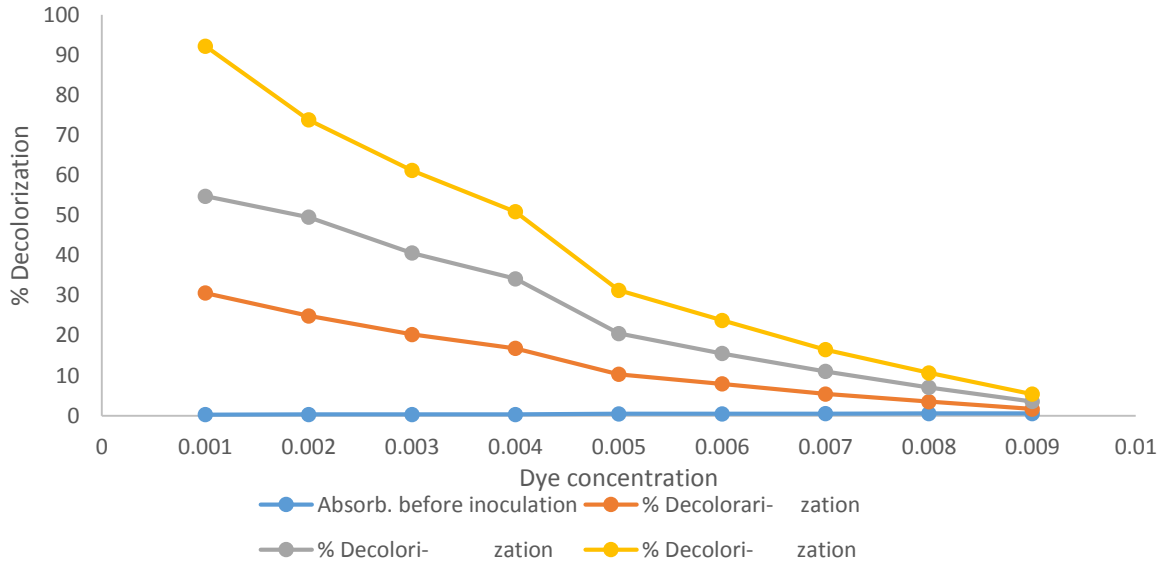

Figure2: Decolourization of Thiazole Dye using *Aspergillus Niger*.

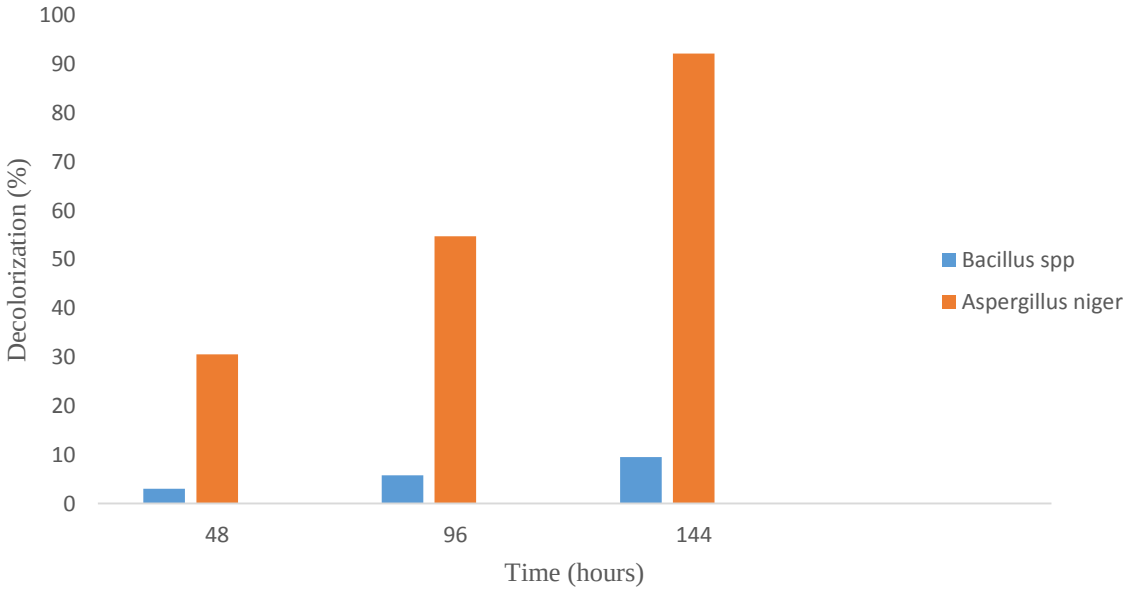

Figure 3: Summary of Microbial Decolourization of Thiazole dye Using Bacillus and Aspergillus niger

Effect of pH on dyeing on Dyeing Performance

Dyeing of the gloving leather was carried out between the acidity range of pH 5.0 -5.5 where maximum absorption was achieved and subsequently fixed at pH 3.8 – 4.0 using a popular formic acid [11]. Further increase in pH was observed to lower the colour strength possibly due to hydrolysis factor [18], which madethe colour of the resultant leather dull. The highest percentage exhaustion value of the dyebath was 81.61% recorded at pH 3.0. However, the dyeing process was taken beyond the conventional pH as the substrate was still absorbing the dye even at pH value further than 8.0 as shown in Table 1.0. Meanwhile, decrease in percentage exhaustion with increase in pH was observed, which is in agreement with the conventional procedures [11, 19]. Light fastness properties were observed from fairly good to good at all pH levels (3-8) but, wash fastness and perspiration properties were not impressive as indicated in Table 5.0, most probably because of the effects of pH. Dyeing of leather is often carried out between pH 5- 5.5 and its fixation is optimal within the pH of 3.8 -4.0 for acid dyes. This suggests reasons for the behavior of perspiration and wash fastness property of the dye on leather fiber as shown in Table 5.0.

Effect of Temperature on Dyeing Performance

Effect of temperature on dyeing shows a steady increase in percentage absorption and subsequent exhaustion with corresponding increase in temperature as presented in Table 2.0. The percentage exhaustion increased from 33.72% to a maximum of 69.76% at 55^{o}C which is the optimal dyeing temperature for tanned leathers [20]. But as temperature increased the substrate was observed to absorbed more dyes leading to more exhaustion of the dyebath, but the leather is at risk of shrinkage and may be deformed at temperatures beyond 60^{o}C [11, 20, 21]. Similar works have also been reported by other researchers [20, 22]. The effect of temperature increase favours absorption of dyes by the leather fiber, particularly as the substrate has abundant reactive sites for the substance. Percentage exhaustion and effects on fastness properties were observed to have been influenced by the effect of temperature.

For wet and dry rubbing, fastness was observed to be good and no significant difference between the two properties as shown in Tables 6, 7 and 8. But its fastness properties at pH 5.0 – 11.0 may be difficult to understand. The effects of increase temperature on the effects of wet and dry rubbing and the fastness properties of the gloving leather did not show any significant difference

on both light, wash and perspiration fastness properties, even when the rubbing was extended to 128 revolutions and temperature of 60ºC, Tables 9 – 11.0.

Effect of Concentration on Dyeing Performance

Effect of Concentration on dyeing performance as indicated in Table 3.0indicated that increase in the concentration of the dye decreased its percentage exhaustion at a point. As concentration increasedfrom 0.002% to 0.012%, the number of dye molecules available for the substrate in the dye bath increased. Leather has abundant reactive sites that will continue to absorb dye molecules especially at optimal pH and temperatures[20, 21]. The continuous percentage exhaustion could be attributed to excellent absorption of the dyes by the substrate as temperature increased thereby leaving little or no dyes in the effluent making fixation excellent and subsequently the exhaustion. Thus concentration at 0.012% had percentage exhaustion of 29.07% while that of 0.002% had 70% exhaustion leaving a clear dyebath effluent. However, exhaustion is not proportional to the dye concentration or absorbance as suggested in Table 2.0. Exhaustion is influenced by the fixing ability of the acid as it will continue to charge the amino group of the collagen for more dye molecules to have fixation sites and consequently the exhaustion. However, there is limitation because, when the acid is in excess, dissolution of the dye from the substrate will commence a situation known as bleeding[20,21]. However, effects of concentration on light fastness property was excellent all through, most properly because the concentration was high to have increased the colour intensity hence was not affected by light. Wash and perspiration fastness were also observed to be good at higher concentrations Tables 10.0 and 11.0.

Decolourization of Thiazole dye bath Wastewater

Figure 1.0 shows the percentage decolourization of the dye bath after 144 hoursof inoculation and agitation with *Bacillus Spp.* Decolourization was observed to decrease with increase in the concentration of the dye in the dye bath wastewater. An example can be seen from 0.001% concentration having the highest decolourization value of 9.5% at 37ºC to 0.7% decolourization of dye bath wastewater with 0.009% concentration. The decolourization is most effective with less concentrated dyebath after 144 hours of inoculation and treatment. The values are however higher when compared with the values obtained for decolourization using Aspergillus niger as shown in figure 2.0. The lowest dye concentration has the highest decolourization value at 92.2% at room temperature after 144 hours of agitation using *Aspergillus niger*. The result obtained

confirms the reports that *Aspergillus niger*is capable of degrading azo dyes [23, 24,25] and [26,27, 28]. This is clearly shown in figure 3.0 which indicates a summary of the decolourization ability of the microorganisms with respect to the duration of treatment.

CONCLUSION

The application of synthetic thiazole dye for dyeing of fancy gloving leather was found to be effective as it was observed to impact very good usage properties. The wastewater generated during the dyeing process was treated with *Bacillus spp*and *Aspergillus niger*. The result obtained suggests that both microorganisms have prospects to degrade the dye bath wastewater but *Aspergillus niger* was found to have better degrading potentials of decolourization of the dyed wastewater.

RECOMMENDATION

Since various physicochemical parameters studied in this experiment influences the decolourization performance of the behavior of these microorganisms, optimization of these parameters is therefore necessary to obtain an optimal condition. Themicroorganism with the greater decolourization performance should be tested using a large scale treatment system to examine its potential for the degradation of dye-polluted wastewaters from either tannery or textile industry.

REFERENCES

1. Tripathi, A. & Srivastava, S.K. (2011).Eco-friendly Treatment of Azo Dyes: Bio decolourization of Bacterial Strains. *Int. J. Biosci. Biochem. &Bioinfor* ,1(1), 32 – 39
2. Praveen, K.G.N. & Bhat, S. K. (2014). Decolourization of Azo Dye Red 3BN by Bacteria. *I. Res. J. Biological Sci.* 1(5), 46-52.
3. Lone, T.A., Revathi, C. & Lone, R. A. (2015). Isolation of Dye Degrading *Bacillus* Species from the Soil near Dyeing Industry and its Potential Application in Dye Effluent Treatment. *Amer-Euras. J. Toxicol. Sci.*,7 (3), 129-135.
4. Franciscon, E., Zille, A., Dias, G. F., Ragagnin de Menezes, C., Durrant, L. R.,& Cavaco-Paulo, A. (2009). Biodegradation of textile azo dyes by a facultative Staphylococcus arlettae strain VN-11 using a sequential microaerophilic/aerobic process. *Inter. Biodeter. & Biodegrad* ,63, 280–288.
5. Shakir, L., Ejaz, S., Ashraf, M., Aziz, Qureshi, N., Ahmad Anjum, A., Iltaf. (2012). Eco

toxicological risks associated with tannery effluent wastewater. *Environmental Toxicalpharmacol*, 34, 180 -191.

6. Saranraj, P. (2013). Bacterial biodegradation and decolourization of toxic textile azo dyes. *Afr. J. Micro. Res.* 7(30), 3885-3890.
7. Robinson, T., McMullan, G., Marchant, R. & Nigam, P. (2001). Remediation of dyes in textile effluent: a critical review on current treatment technologies with a proposed alternative (review), *Biores. Techno.*, 77(3), 247-255.
8. Shah, M. P. (2014). Biodegradation of Azo Dyes by Three Isolated Bacterial Strains: An Environmental Bio-remedial Approach. *J. Microbial Biochem. Technol.*, S3: 007. doi:10.4172/1948-5948.S3-007
9. Dixit, S., Yadar, Ashish, P., & Duriredi, M. (2014). Toxic hazards of leather industry and technology to combat threat, *Journal of cleaner production* pp 39 – 49
10. Ezhilarasu, A. (2016). Textile industry Dye degrading by bacterial strain *Bacillus* spp.*Int. J. Adv. Res. Biol. Sci.*3 (3), 211-226.
11. Sarkar, K.T. (2005). Theory and Practice of Leather Manufacture. *Published by the author, NeelachalAbasan 98, Rajdanga Gold Park Kolkata. 72 -128*
12. SLTC (2014a). IUF 423, International Union of Physical Testing IUP/421: Official Method of Analysis.*J. Soc. Leather Technol. Chem. 82, 185- 1189*
13. SLTC (2014b). IUF 423, Determination of Wash Fastness Property: Official Method of Analysis.*J. Soc. Leather Technol. Chem. 82, 200.*
14. Abioye, O.P., Iroegu, V.T. and Aransiola, S.A. (2015).Biodegradation of Methyl Red by *Staphylococcus Aureus* Isolated from Waste Dump Site. *J. Environ. Sci. Technol.* 8 (3), 131-138.
15. SLTC (2014c). IUF 420, Determination of Light Fastness Property: Official Method of Analysis. *J. Soc. Leather Technol. Chem. 82, 200.*
16. SLTC (2014 (d). IUF 420, Determination of Perspiration Fastness Property: Official Method of Analysis. *J. Soc. Leather Technol. Chem. 82, 205 -207.*
17. Pepper, I. L. and Gerba, C. P., (2004). *Environmental Microbiology*. A Laboratory Manual 2nd edition, Printed in U.S.A
18. Banat, I.M., Nigam, P., Singh, D. & Marchant, R. (1996). Microbial decolorization of textile dye containing effluent: A review. *Biores. Technol.* 58, 217-227

19. Ahmad, B., Bhatti, I.A., Saeed, Q. & Abbas, M. (2012). Synthesis and Applications of three Vinylsuphone Based Fibre reactive azo Dyes for Dyeing Cotton Fabric. *Int. J. Basic & App Sci.*, 12(6), 129-136.

20. Covington, T. (2009) Tanning chemistry: The science of Leather, published by the Royal Soceity of Chemistry. Thomas Graham House Science Park Milton Road Cambridge CB4 OWF, UK 112 – 154.

21. Leafe, M.K. (1999). Leather Technologist Pocket Book. Published by Society of Leather Technologist and Chemist, 204 Queens street Withernsea East Yorkshire, *HU19 2NR* U K. 29 - 34

22. SLTC (2014 (e). IUF 420, Determination of Fastness Property due to wet and dry rubbing: Official Method Analysis.*J. Soc. Leather Technol. Chem. 82, 189 -201;*

23. Ezeribe A. I., Bello K. A., Adamu H. M., Chindo I, Y. & Boryo D, E, A. (2013). Synthesis and Dyeing Properties of Novel Bifunctional Reactive Dyes Via 4-(4-Methoxyphenyl)-Thiazol-2-Amine and 4-(4-Bromophenyl) -1, 3- Thiazole- 2- Amine On Nylon Fabric, *The Int. J. Eng. & Sci. (IJES),* 2(8), 2013.

24. Fu, Yuzhu & Viraraghavan, T (2001). Fungal decolourization of dye wastewater: a view.Bioresource Technology,73(3) 251 -262. Doi:10.1016/S0960 – 8524(01)00028-1

25. Wesenberg, D. Kyriakides, I. & Hyathos, S. N. (2003). White-rot fungi and their enzymes for the treatment of industrial dye effluents. Biotehnology Advances, 22 (2),161 – 187. Doi:10,1016/jibiotechadv.2003.08.011

26. Sudha, M., Saranyal, A. Selvakumar, G. & Sivakumar, N. (2014). Microbial degradation of Azo Dyes: Inter. J. curr. Microbiol. App. Sci 3 (2) 670 – 690.

27. Anjeli, P., Poonam, S. & Leela, I. (2007). Bacterial decolourization and degradation of azo dyes. *Int. J. Biodeter. &Biodegrad.* 59 (2), 73-84.

28. Shahid, M., Muhammad, A., Azeem, K., Zilli, H.N. & Tariq, M. (2011). Isolation and screening of azo dye decolorizing bacterial isolates from dye-contaminated textile wastewater. *Soil Environ.* 30(1), 7-12.