International Journal of Students Research in Technology & Management
Vol 1 (04), August 2013, ISSN 2321-2543, pg 370-382

EVALUATION OF EFFICACY OF BACTERIAL CONSORTIUM B3RVY 3 FOR REMOVAL OF TOXICITY FROM DIFFERENT TEXTILE DYES

Hemangi Patel and Darshan Marjadi

Department of Biotechnology, Shree Ramkrishna Institute of
Computer Education and Applied Sciences, Surat-395001, Gujarat.
patel.hemangi91@gmail.com

Abstract

Toxic effluents containing synthetic dyes are discharged from various industries and they adversely affect water resources, soil fertility, aquatic organisms and ecosystem integrity. In this study, a novel bacterial consortium isolated from dye contaminated soil sample was shown to effectively decolorize Reactive Violet 5 and Golden Yellow HER within 22 hrs. under static condition at a maximum dye concentration of 400 mg/L and was named as B3RVY 3. Biochemical analysis and 16S rRNA sequencing was done for identification of the bacterial isolates responsible for degradation of the dyes. Dye decolorization was monitored using scanning Ultraviolet-Visible spectrophotometer which indicated that decolourization was due to the degradation of dyes into non-colored intermediates. The protein content estimation in raw dye and its degradation metabolites was performed and it was found that protein content increases with the increase in the concentration of the dye. Thus the stress is the main reason that shows the influence of protein content and responsible for degradation. Phytotoxicity study of both dyes on *Vigna radiata* and *Triticum aestivum* revealed the more toxic nature of raw dye and less toxic nature of its degradation metabolites. Microbial toxicity study on *Escherichia coli* and *Staphylococcus aureus* also showed the more toxic nature of raw dye and less toxic nature of its degradation metabolites.

Keywords: Synthetic dyes, Biodegradation, Bacterial consortium B3RVY 3, 16S rRNA sequencing, Phytotoxicity, Microbial toxicity

I. INTRODUCTION

Dyes are the colored substances which fixes firmly with the substrate. Nowadays, one of the most developing industries is textile industry for the development of colored fabrics. Synthetic dyes are extensively used in a number of industries such as textiles, paper printing, colour photography, and the food industry. A major class of synthetic dyes includes the azo, anthroquinone and triphenylmethane dyes. A number of dyes and auxiliary chemicals used in textile and dyeing industries are producing strongly coloured effluents which has now become of critical environmental concern (Patel *et al.*, 2013). Inefficiency of the dyeing process, poor handling of spent effluent and

insufficient treatment of wastes of the dyestuff industries lead to dye contamination of the environment such as soil and natural water bodies. These pollutants are released into water and soil and have detrimental effects on the health of humans, plants, animals and microbes. Thus, the wastewater must be treated before releasing into the natural environment. A number of physicochemical methods, such as adsorption, coagulation, precipitation, filtration and oxidation, have been used to treat dyestuff effluents, but these methods have many disadvantages and limitations. It is, therefore, important to develop efficient and cost-effective methods for the decolourization and degradation of dyes in industrial effluents and contaminated soil. In particular, systems based on biological processes using a large variety of bacterial strains, allow for degradation and mineralization with a low environmental impact and without the use of potentially toxic chemical substances, under mild pH and temperature conditions (Dhanve *et al.*, 2008, Khalid, *et al.*, 2008**).**
Biological treatment is considered to be the most effective treatment option for the treatment of dying industry wastewater. Activated sludge is commonly used as an inoculum to initiate degradation, and it appears that many different microorganisms can decolorize azo dyes (Pandey *et al.*, 2007) but may require a mixed community to mineralize them. Amongst these systems, several microbial strains including *Sphingomonas spp., Pseudomonas luteola, Streptococcus faecalis, Klebsiella pneumoniae, Penicillium spp., Aspergillus spp.* have been described as being capable of reducing azo dyes (Hsueh and Chen, 2008). These microorganisms have the ability not only to decolourise dyes but also to detoxify it (Kumar *et al.*, 2006). Furthermore, it has been demonstrated that local indigenous microflora biomass is significantly better at biodegradation/ biodecolourization than commercially obtained.

The objective of this study was to focus our attention on the isolation of dye-decolourizing microorganisms from contaminated soil of an industrial estate and reporting a novel consortium named B3RVY 3 having decolourizing ability for Reactive Violet 5 and Golden Yellow HER. UV-visible spectrophotometer analysis was done to confirm that decolourization was due to degradation of dyes into non-colored intermediates. The toxicity of raw dye and the degradation metabolites formed after decolorization was determined using phytotoxicity and microbial toxicity assay.

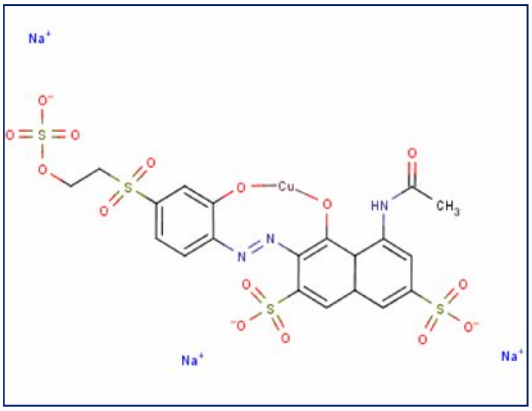
II. MATERIALS AND METHODS

Chemicals:

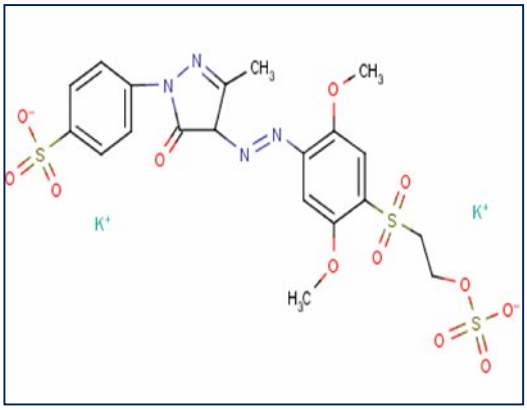
All chemicals used were of analytical grade. The common name of both dyes has been used for convenience; the dyes were collected form textile industry, Surat, India. The dyes were Reactive Violet 5(C.I. no 18097) and Golden Yellow HER (C.I. no 18852). Bushnell Hass Medium, Glucose and yeast extract were obtained from Sisco Research Laboratories, Andheri, Mumbai, India. Bovine Serum Albumin (Loba chemie Pvt. Ltd, Colaba, Mumbai, India), Folin and Ciocalteu's were purchased from Sisco Research Laboratories, Andheri, Mumbai, India.

Table 1: Chemical structure and characteristics of azo dyes

Name	C.I No.	λ_{max}	Molecular weight
Reactive violet 5	18097	560	735.58
Golden yellow HER	18852	530	682.77



Reactive violet 5



Golden yellow HER

Medium:

The microbial consortium B3RVY 3 was routinely grown at 37 C^0 in basal culture medium containing Bushnell and Hass medium (BHM) containing the following in g/l: $MgSO_4$, 0.2; K_2HPO_4, 1.0; $CaCl_2$, 0.02; $FeCl_3$, 0.05; NH_4NO_3, 1.0 supplemented with glucose (0.1% w/v) and yeast extract (0.05% w/v).

Isolation of bacterial cultures:

Soil samples collected from contaminated sites around dye industries in Surat industrial estate , Gujarat were used for screening of dye-decolourizing cultures by enrichment techniques using BHM amended with dye (RV 5 and GY HER, 400 mg/l) along with glucose and yeast extract as co-substrates. Dye-containing media (100 ml) in 250 ml Erlenmeyer flasks were inoculated with 3 ml (3%) of soil suspension and incubated under shaking (150 rpm) and static conditions at 37^0 C. Consortia which showed decolourization in liquid media were tested further by transfer in fresh dye containing media for consistence decolourization in every successful transfer.

Dye decolourization measurement:

Decolourization study of Reactive Violet 5 and Golden Yellow HER was carried out by UV-Visible spectrophotometer analysis. The BHM medium containing Reactive Violet 5 (400 mg/l) and Golden Yellow HER (400 mg/l) was inoculated with 3% microbial culture and incubated under static condition at 30^0 C. The 2 ml of culture was withdrawn at 0, 5, 10, and 15 hours and centrifuged at 8000 rpm foe 5 minutes. The residual dye concentration in supernatant was measured for Reactive

Violet 5 at 560nm and Golden Yellow HER at 530nm. The absorbance of microbial cell mass responsible for decolorization of RV 5 and GY HER dyes were observed at 660nm.

The percentage of dye decolourization was calculated by the following formula:

% of decolourization = (Control O.D – sample O.D × 100) / Control O.D.

Total protein content analysis:

Estimation of total protein was carried out by Folin's Lowry's method.

Extraction of proteins:

The culture (10 ml) was taken in clean autoclaved falcon tubes and centrifuged at 8000 rpm for 5 minutes at 4^0C. After centrifugation, the supernatant was collected in a new falcon tube. The supernatant was used for the estimation of protein.

Procedure:

To 1, 2, 3 and 4 ml of standard BSA solution 4, 3, 2 and 1ml of distilled water was added to make final volume 5 ml. The unknown protein solution was diluted (1:5) and final volume was made up 5 ml with distilled water. To each tube, 5 ml alkaline copper solution reagent was added and, mixed thoroughly and incubated at room temperature for 5 minutes. To each tube, 5 ml alkaline copper solution reagent was added and, mixed thoroughly and incubated at room temperature for 5 minutes. 0.5 ml folin –ciocalteu reagent was then added in each tube, mixed well and incubated in dark for 30 minutes. After incubation, the O.D of each tube was determined at 750 nm.

Phytotoxicity study:

Phytotoxicity tests were conducted to assess the impact of Reactive Violet 5 and Golden Yellow HER and its degradation metabolites on vegetation and to explore the possible reuse of the treated solution in the irrigation of agriculture field. Tests were carried out on two kinds of plants which are most sensitive, fast growing, and commonly used in Indian agriculture: *Vigna radiata* and *Triticum aestivum.* First the test was conducted to determine LD_{50} and highest toxic concentration of both dyes by placing 10 seeds of each plant in raw dye solution at a concentration of 50,100,200,300, and 400 mg/l of both dyes. After that, 10 seeds of each plant were placed in each Petri dish in sets and watered separately with 10 ml samples at a LD_{50} concentration and highest toxic concentration of both dyes and degradation products of LD_{50} and highest toxic concentration of both dyes per day. Control set was carried out using tap water at same time. % Germination, Seedling dry Weight, length of plumule (shoot) and radical (root), length of leaf and seedling length were recorded after 7 and 14 days.

Microbial toxicity study:

For the purpose of assessing the impact of Reactive Violet 5(300 and 400 mg/L), its degradation metabolites (300 and 400 mg/L), Golden Yellow HER (100 and 400 mg/L) and its degradation metabolites (100 and 400 mg/L) microbial toxicity tests were performed on the microorganisms

Escherichia coli and *Staphylococcus aureus*. The microbial toxicity assessment was evaluated based on agar well diffusion technique. Test microorganism cultures were grown in nutrient broth. Test organisms were then inoculated in 1.5% top agar and poured on 3% previously solidified nutrient agar (base agar). A flame sterilized cork borer with a diameter of 5 mm was used to bore 4 wells into agar and100 µl of control of both dyes (LD_{50} and highest concentration) and its metabolites (of LD_{50} and highest concentration) was loaded into wells. The dye and extract was allowed to diffuse into agar before plates were incubated under aerobic conditions at 37^0C for 24 hr. At the end of the incubation period, plates were observed for zones of inhibition around the wells. Inhibition zone is defined as the area free of growth in a bacterial lawn which results from the toxic effect of compounds that has diffused into the medium from its applied source.

III. RESULTS AND DISCUSSION:

Isolation of bacterial cultures:

Isolation from soil sample were carried out by enrichment culture technique using RV 5 and GY HER as a sole source of carbon and energy. But we were not successful in isolating bacteria capable of decolourizing and utilizing only dyes as sole source of carbon and energy. Decolourization occurred only when a carbon and energy sources were available in the growth medium .Using BHA with glucose and yeast extract along with RV 5 and GY HER we isolated three different consortia from three different soil samples capable of decolourizing RV 5 and GY HER under static conditions. Of all the consortia obtained, bacterial consortium B3RVY 3 was able to degrade Reactive violet 5 and Golden Yellow HER at a wide range of temperature 35-40^0 C and decolourization at alkaline pH. The culture showed dye degradation by varying concentration from 100mg-400mg/L but at higher concentration, rate decreases with increase in toxicity. The culture showed degradation of Reactive violet 5 within 20 hours whereas Golden Yellow HER was degraded by culture within 15 hours. Complete degradation (100%) of both dyes was occurred within 22 hours.

The decolourization of Reactive Violet 5 was also reported by safia moosvi. Of all consortia obtained, the bacterial consortium RVM 11.1 was found to be most efficient with maximum decolourization (94%) efficiency when incubated under stationary conditions at 37^0 C. Parshetti *et al.* 2007 reported that *K. rosea* showed 100% decolourization of Malachite Green (50 mg/L) within 5 h at static anoxic condition whereas there was no decolourization at shaking condition. The reason for no decolourization at shaking condition could be competition of oxygen and dye for the reduced electron carriers under aerobic condition. There was no abiotic loss of Malachite Green within 5 h of incubation. It has been shown that the cultures of *C. elegans* transformed apparently 85% of MG (81 mM) in culture flasks within 24 h. A concentration of 108 mM Malachite Green inhibited fungal growth and biotransformation did not occur. This could be first report on 100% decolourization of Malachite Green within 5 h at 50 mg/L concentration by *K. rosea*.

Dye decolourization measurement:

The variation in decolourization efficiency of isolates may be attributed to differences in the chemical structure of the dye and the varying metabolic function of the different bacterial isolates. UV-visible spectral analysis was used to confirm that the decolourization process of the azo dyes was due to biodegradation process. If the dye removal is attributed to biodegradation, either the major visible light absorbance peak would completely disappear or a new peak will appear. The absorbance peak (at 0 h) of the dyes drastically reduced at different rates within 15 hours of incubation. No new absorbance peak appeared in samples after scanning decolourization which indicates the breakdown of the dyes to non absorbing metabolites. Ultraviolet–visible scan (400–800 nm) of culture supernatants withdrawn at different time intervals indicated decolourization and decrease in dye concentration from batch culture. Peak observed at 440 nm (0 h) was decreased without any shift in λ_{max} up to complete decolourization of dye (8 h) (Thomas Sawidis, 2011).

UV- Visible spectral analysis was used to confirm that the decolourization process of the RV 5 and GY HER, azo dyes was due to biodegradation. The B3RVY 3 culture showed complete decolourization of Reactive Violet 5 [400(mg/L)] and Golden Yellow HER [400 (mg/L)] within 22 hours at static anoxic conditions. The percentage of decolourization decreases with increase in dye concentration. Ultraviolet-Visible scan (560nm) of culture supernatant and scan (660nm) of biomass harvested at different time intervals indicated decolourization and decrease in RV5 dye concentration (Fig 1). Ultraviolet-visible scan (530nm) of culture supernatant and scan (660nm) of biomass harvested at different time intervals indicated decolourization and decrease in GY HER dye concentration (Fig 2).

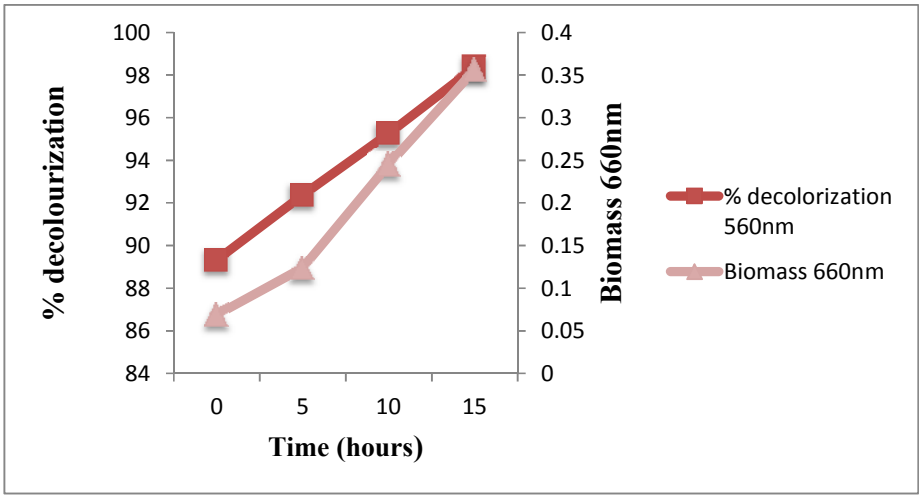


Figure 1: % decolourization of dye versus biomass of Reactive Violet 5 by B3RVY 3 culture

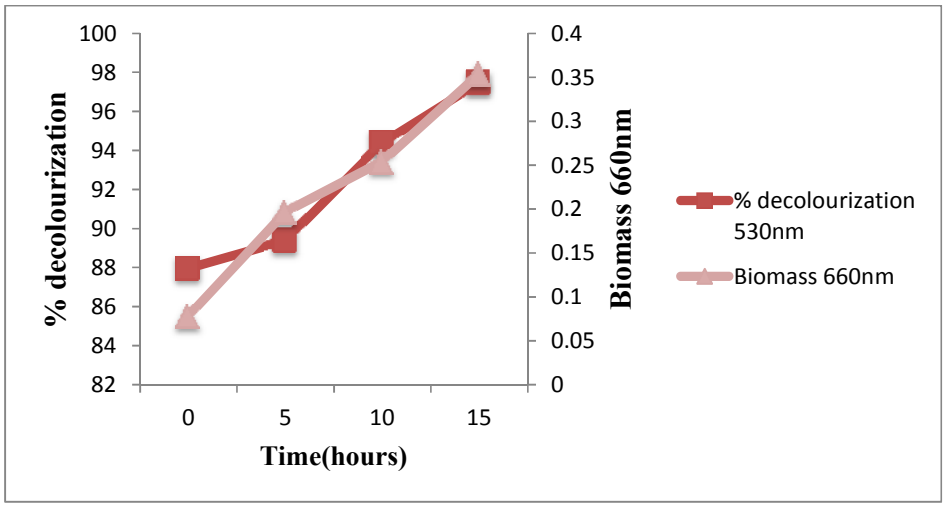


Figure 2: % decolourization of dye versus biomass of Golden Yellow HER by B3RVY 3 culture

Total protein content analysis:

The unknown protein content can be quantified by plotting the optical density values in the BSA standard graph. The absorbance values of the BSA with respect to the concentration of the protein were measured using UV spectrophotometer at 750nm (Table 2). The quantification of total unknown protein content present in degradation metabolites was done by plotting the optical density values in the Standard plot (Table 3). The results showed that the concentration of the protein increases with the increases in the concentration of dye. Thus, it clearly indicated that the stress (dye) shows the influence of the protein content.

Table 2 Standard BSA plot values

Concentration of BSA (µg/ml)	Optical density at 750nm
20	0.064
40	0.186
60	0.374
80	0.492

Table 3 Total protein content in dye solution after degradation

Dye	Concentration of dye(mg/L)	Concentration of protein(µg/ml)
Reactive Violet 5	300	54

Reactive Violet 5	400	57
Golden Yellow HER	100	60
Golden Yellow HER	400	70

It was found that protein concentration with Golden Yellow HER dye was quite higher than that of Reactive Violet 5 at both the concentration of dyes. It was also noticed that there was no significance difference in protein content at two different concentrations of both the dyes.

Phytotoxicity study:

Toxicity assay was applied to evaluate the phytotoxicity on plants based on the germination percentage of seeds. The germination percentage combines measurements of relative root and shoot elongation as both are sensitive to the presence of phytotoxic compounds. The results of determination of LD_{50} was 100 (mg/L) of Golden Yellow HER and 300(mg/L) of Reactive Violet 5. Phytotoxicity study revealed the toxicity of Reactive Violet 5 [LD_{50} (300 mg/L) and highest toxic concentration (400 mg/L)] to the *Vigna radiata* and *Triticum aestivum* plants and Golden Yellow HER [LD_{50} (100 mg/L) and highest toxic concentration (400 mg/L)] to the *Vigna radiata* and *Triticum aestivum* plants. Germination percentage of two plants was less with Reactive Violet 5 and Golden Yellow HER treatment as compared to metabolites of both LD_{50} and highest toxic concentration obtained after its decolourization and distilled water treatment Reactive Violet 5 and Golden Yellow HER both dyes were significantly reducing the length of plumule and radical in *Vigna radiata* and than its metabolites. Phytotoxicity study revealed the toxic nature of Reactive Violet 5 and Golden Yellow HER and less toxic nature of the metabolites obtained after decolourization of Reactive Violet 5 and Golden Yellow HER.

Amongst the dyes, treatment Golden Yellow HER showed the most toxic effect on seed germination and plant growth parameters of both plants whereas, treatment with Reactive Violet 5 exhibited the least toxic effect. However, for the dye metabolites, treatment with Golden Yellow HER showed the most toxic effect while Reactive Violet 5 exhibited the least toxic effect. Results indicate that effective dye decolourization does not always result in reduction of dye toxicity since Golden Yellow HER was better decolorized than Reactive Violet 5. However, toxicity exerted by the treated samples was generally lower than that obtained for the untreated samples. Before now, most of the decolourization projects have concentrated mainly on colour removal while neglecting the fact that sometimes biological processes are connected with formation of toxic intermediates. Hence, it is required that the evaluation of decolourization effect be carried out with relation to eco toxicity assessment.

Table 4 Toxic effect of Reactive violet 5 on *Vigna radiata*

Observation	Tap water	300(dp) (mg/L)	300(org) (mg/L)	400(dp) (mg/L)	400(org) (mg/L)
Germination%	98.66	78	56.33	66	45.66
Plumule length(cm)	11.5	7.46	5.26	5.21	4.51
Radical length(cm)	1.9	1.73	1.1	1.2	0.87
Seedling dry weight(gm)	0.057	0.052	0.042	0.041	0.034

Table 5 Toxic effect of Golden Yellow HER on *Vigna radiata*

Observation	Tap water	100(dp) (mg/L)	100(org) (mg/L)	400(dp) (mg/L)	400(org) (mg/L)
Germination%	99.33	81	76	65	47
Plumule length(cm)	9.4	6.53	6.35	5.22	4.51
Radical length(cm)	1.2	0.61	0.44	0.51	0.19
Seedling dry weight(gm)	0.060	0.055	0.049	0.044	0.039

Table 6 Toxic effect of Reactive Violet 5 and on *Triticum aestivum*

Observation	Tap water	300(dp) (mg/L)	300(org) (mg/L)	400(dp) (mg/L)	400(org) (mg/L)
Germination%	99.66	75	70	73	66.6
Plumule length(cm)	3.36	2.34	2.28	1.62	1.5
Radical length(cm)	1.1	0.81	0.69	0.73	0.38
Seedling dry weight(gm)	0.065	0.057	0.054	0.053	0.050

Table 7 Toxic effect of Golden Yellow HER on *Triticum aestivum*

Observation	Tap water	100(dp) (mg/L)	100(org) (mg/L)	400(dp) (mg/L)	400(org) (mg/L)
Germination%	90	71	20	56.6	10
Plumule length(cm)	5.14	4.21	0.11	1.9	0.10

Radical length(cm)	0.94	0.49	0.10	0.4	0.10
Seedling dry weight(gm)	0.048	0.046	0.030	0.031	0.027

Phytotoxicity study (Parshetti *et al.*, 2011) revealed the toxicity of Crystal Violet to the *Sorghum bicolor*, *Vigna radiata*, *Lens culinaris*, and *Triticum aestivum* plants. Germination percentage of all four plants was less with Crystal Violet treatment as compared to metabolites obtained after its decolourization and distilled water treatment. Crystal Violet was significantly reducing the length of shoot and root than its metabolites. Phytotoxicity study revealed the toxic nature of Crystal Violet and less toxic nature of the metabolites obtained after decolourization of Crystal Violet.

Moawad *et al.* (2003) reported the phytotoxicity of different soluble textile dyes estimated by measuring the relative changes in seed germination of four plants: clover, wheat, tomato and lettuce. Dawkar *et al.* (2007) reported phytotoxicity of Brown 3REL.The mean of plumule and radical length of *Triticum aestivum* was 9.2 cm (±0.02) and 9.5 cm (±0.06) respectively of the 10 seeds in distilled water as a control with 100% germination and for Sorghum biocolor was 10.2 cm (±0.02) and 9.8 cm (±0.04). The seed germination was completely inhibited, when seeds were treated with 4000 ppm concentration of Brown 3REL, where as the plumule length and radical length were found to be 9 cm (±0.13) and 9.3 cm (±0.07), respectively when treated with 4000 ppm degradation product (100% germination) and for *S. biocolor* was 10 cm (±0.17) and 9.5 cm (±0.09). This indicates the less toxic nature of the degradation product to the plants.

Microbial toxicity study:

Microbial toxicity studies revealed that zone of inhibition was observed with Reactive Violet 5 (400mg/L) and Golden Yellow HER (400mg/L) by both bacterial strains studied, whereas, its metabolites showed comparatively less zone of inhibition. These findings suggest that the degradation products were less toxic compared with the Reactive Violet 5 (400mg/L) and Golden Yellow HER (400mg/L) to an exploited microorganism's *Escherichia coli* and *staphylococcus aureus*.

The results of the study of microbial toxicity of crystal violet (Parshetti *et al.* ,2011) showed that the degradation products were less toxic compared with the Crystal Violet to an exploited microorganism's *A. radiobacter*, a phosphate solubilizing bacterium *P. aeruginosa* and nitrogen-fixing bacterium *A. vinelandii*. Zone of inhibition was observed with control Malachite Green with all bacterial strains studied whereas its degradation products did not show growth inhibition. These findings suggest non-toxic nature of the product formed. Previous reports showed MG degradation into leucomalachite green that is equally toxic to malachite green (Parshetti *et al.* 2007).

Table 8 Microbial toxicity studies of Reactive Violet 5 and Golden Yellow HER and metabolites obtained after its decolourization

Bacteria	Reactive Violet 5 (mg/L)	Diameter of zone of inhibition (mm)	Golden yellow HER (mg/L)	Diameter of zone of inhibition(mm)
E. coli	400 (org) 400(dp) 300(dp)	30 N.I N.I	400(org) 400(dp) 100(dp)	28 N.I N.I
S.aureus	400 (org) 400(dp) 300(dp)	40 N.I N.I	400(org) 400(dp) 100(dp)	32 N.I N.I

N.I.: no inhibition

IV. CONCLUSION

The results showed significant decolourization and degradation of Reactive Violet 5 and Golden Yellow HER by the bacterial consortium B3RVY 3. In this study it was found that the isolation and characterization of bacterial species present in B3RVY 3 culture capable of efficiently degrading azo dyes Reactive Violet 5 and Golden Yellow HER. The results of total protein content analysis clearly indicated that the stress (dye) shows the influence on the protein content. Phytotoxicity tests were carried on two plant species *Vigna radiata* and *Triticum aestivum* also indicated detoxification of the dyes after degradation as decolorized samples of both LD_{50} and highest toxic concentration exhibited lower toxic effect than the raw dyes. Toxicity tests on *E. coli* and *S. aureus* also indicated the lower toxic effect of dye after degradation than the raw dye.

V. FUTURE PERSPECTIVES

Biodegradation of synthetic dyes using different fungi, bacteria, yeasts, and algae is becoming a promising approach for the treatment of dye wastewaters. Green plants are nature's factories, which fix inorganic chemicals (CO_2 and H_2O) into organic form (glucose and then other complex molecules) through photosynthesis and other reactions, while microbes are the nature's tools, which convert back the organic materials (dead bodies of plants and animals) to inorganic form (CO_2, H_2O, and salts) through decomposition and mineralization. Thus, green plants and microbes are responsible for keeping a balance between the organic and inorganic worlds. With the increasing production of synthetic chemicals and their ultimate release into the environment, the natural microbial populations

are unable to decompose them in due course of time. As a result, such chemicals are accumulated in the ecosystem and affect the quality of life. Keeping in mind the increasing production of these chemicals and their persistence in the natural environment, their removal is utmost necessary. By exploiting the biodegradation potentials of different microbes, it is possible to handle the problem in a better way. A better understanding of biodegradation of synthetic dyes requires knowledge of chemistry and microbiology, whereas its application on industrial scale requires knowledge of biochemical engineering as well. Thus, research in this field is highly interdisciplinary in nature. Now, since interdisciplinary research is highly encouraged and valued worldwide especially in broad minded communities, it is fully hoped that the science and technology of biodegradation of organic xenobiotics will emerge as a leading one for control of environmental pollution. An understanding and knowledge of biodegradation are not only helpful in pollution abatement but also in the production of biofriendly and environment friendly products like biodiesel, bioethanol, biopesticides, biopolymers, etc. The biodegradation abilities of microorganisms can be enhanced by gradually exposing them to higher concentrations of synthetic organic chemicals. Adaptation of a microbial community toward toxic or recalcitrant compounds is found to be very useful in improving the rate of decolorization process (Dafale *et al.*. 2008). The adaptation of microorganisms to higher concentrations of pollutants is called acclimatization and leads to forced or directed evolution. Microorganisms exposed to higher levels of pollutants evolve mechanisms and pathways for handling (degrading) them. This happens through expression of genes encoding for enzymes responsible for degradation. Alternatively, identification, isolation, and transfer of genes encoding for degradative enzymes can greatly help in designing microbes with enhanced degradation capabilities. Thus, acclimatization and genetic engineering both can be helpful in designing super-degraders. Out of the two approaches, acclimatization is natural, since in this case, the built-in genetic setup of the microorganism is not disturbed; only some components are enabled. On the other hand, in genetic engineering, the natural genetic setup of the microorganism is changed by incorporating new gene(s). Therefore, many scientists (especially environmentalists) are sceptic about the usefulness of genetically modified organisms. They fear that such modified organisms will create new environmental problems. Time will prove or disprove the reality of such fears.

VI. ACKNOWLEDGEMENT

I acknowledge the Department of Biotechnology, Shree Ramkrishna Institute of Computer Education and Applied sciences, Surat for their constant support, encouragement, consecutive counsel and unreserved help served as a beacon light throughout the course of study, research work and completion of this manuscript.

REFERENCES

I. Dafale, N., Rao, N. N., Meshram, S. U., & Wate, S. R., **2008.** *Bioresource Technology*, **99**, 2552–2558.

II. Dawkar, V. V., Jadhav, U. U., Ghodake, G. S., & Govindwar, S. P.,**2009**. *Biodegradation,* **20**, 777–787.

III. Dawkar, V. V., Jadhav, U. U., Jadhav, S.U., & Govindwar, S. P., **2007**. *Journal of Applied Microbiology* ISSN 1364- 5072.

IV. Decolorization of azo dyes and simulated dye bath wastewater using acclimatized microbial consortium-Biostimulation and halo tolerance. *Bioresource Technology*, **99**, 2552–2558.

V. Dhanve RS, Shedbalkar UU, Jadhav JP. , **2008**. *Biotechnol Bioproc Eng*., **13**:53–60.

VI. G. K. Parshetti, S. G. Parshetti, A. A. Telke, D. C. Kalyani, R. A. Doong, S. P. Govindwar. *Journal of Environmental Sciences* **2011**, **23(8)** 1384–1393.

VII. Hsueh CC, Chen BY. **2008.154**:703–10.

VIII. Kumar, K., Devi, S.S., Krishnamurthi, K., Gampawar, S., Mishra, N., Pandya, G.H., Chakrabarti, T., **2006**. *Bioresour. Technol*. **97**,407 –413.

IX. Moawad H, Wafaa M, Rahim A E., **2003**. *Fresenius Environmental Bulletin*, **12(7)**: 786–792.

X. Pandey A, Singh P, Iyengar L., **2007**. *Int Biodeter Biodegr* **59**:73–84.

XI. Patel H.R., Marjadi D & Boricha G., **2013**. *International Journal of Chemtech Applications* **Vol. 2; Issue 2**; Page 126-136.