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Effect of various carbon and nitrogen sources on decolorization of textile dye remazol golden yellow using bacterial species

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Abstract

Textile dyes with different chemical structures are consistently used in textile industries and they are being recalcitrant xenobiotic in nature. The aim of present research is directed to finding the preference of striking carbon and nitrogen sources on remazol golden yellow decolorization. Bacterial strains were isolated, screened and tested for dye degradation of remazol golden yellow in basal medium amended with different carbon and nitrogen sources. This study was carried out for the period of 12 d at 37°C. Among various carbon and nitrogen sources, starch and yeast extracts promote maximum decolorization in the medium inoculated with *Bacillus*. sp. (ESL-52). Nevertheless, the rate of decolorization was less in the medium amended with various carbon and nitrogen sources in the presence of *Bacillus* sp. (TSL-9), *Micrococcus* sp. (TSL-7), *Pseudomonas* sp. (M-1) and *Staphylococcus* sp. (ES-37) respectively. The results clearly showed that addition of significant organic carbon and nitrogen sources are only desirable co-substrates for bacterial dye decolorization process.

Key words

Carbon sources, Decolorization, Nitrogen sources, Optimization, Reactive dyes, Remazol golden yellow

Introduction

Textile industry wastes are a major threat to the environment and have attained considerable interest by the environmentalist during the last few decades (Peternel *et al.,* 2007). These textile effluents also contain enormous amount of dyes, surfactants, salts, softeners and auxiliary additives that are non biodegradable and toxic to environmental flora and fauna. The dyes generally resist to photosynthesis process, rapid depletion of the oxygen, unfit for irrigation, increase the BOD and affect the soil texture and permeability. Recent reports have revealed that 12% of synthetic dyes are lost annually in manufacturing unit itself (Srinivasan and Muruthy, 2009).

Worldwide approximately, more than 10,000 dyes and pigments are commercially available in the textile industry (Robinson *et al.,* 2001). Predominately reactive dyes are the largest group of colorants containing different chromophoric groups such as azo, anthraquinone and triarylmethane etc. Azo dyes (–N N–) group are widely used in more than half of the global dye production sectors because of its simple dyeing process and greatest variety of colors. A significant portion of wastewaters generated from these industries constitute major assault to the surrounding ecosystem (Chen *et al.,* 2005). Synthetic dyes from waste water gains more important than other colorless products, mainly due to existence of dyes (below 1 ppm) is clearly visible and affects the nature of water and environment considerably. Hence, there is an urge to find an optimal method for wastewater treatment in order to eliminate colors and other toxic compounds from the textile effluents (Ou *et al.,* 2005).

Currently several physico-chemical technologies such as coagulation with alum, ferric chloride, magnesium carbon, polymer and mineral sorbents or biosorbents chemical oxidation, photolysis, photo catalytic degradation, suspended or supported photocatalysis and electrophotocatalysis are available for the removal of color and COD from azo dye wastewater (Aleboyeh *et al.,* 2005). Unfortunately, all the methods are not economically feasible due to wide range of utilizing chemicals, electricity also to produce large amount of secondary pollutants and sludge. All the

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treatment of wastewater containing dyes is difficult. Traditional methods transfer only the organic group of the contaminants from one phase to another. On the other hand the using microorganism is versatile and useful technique for treatment of dye house effluents (Bi *et al.,* 2009).

The biological treatment of textile wastes using microorganisms is an efficient and appropriate method to degrade recalcitrant contaminants; however, some synthetic dyes are structurally resistant to the biological reactions (Kuhad *et al.,* 2004). Microorganisms play a vital role in the degradation of azo dyes (Syed *et al.,* 2009). Therefore, it is essential to screen dye degrading microorganisms and optimize microbial degradation rate in relation to environmental parameters for the instance effect of co-substrate, pH, temperature, inoculums size, salts and dye concentration (Zhang and Zheng, 2009). Earlier studies stated that microorganisms require additional carbon and nitrogen source for dual functions, such as growth and biodegradation (Jin *et al.,* 2006). It was reported that microorganisms cannot utilize dye as a co-substrate, as it contains toxic compounds. However, few researchers found that bacterial cultures capable of utilizing dyes as carbon or nitrogen sources (Saranaik and Kanekae, 1999). Microorganism requires organic carbon and nitrogen sources for the degradation of dye since they cannot utilize dye as the growth substrate.. The present paper aimed to study the effect of various carbon and nitrogen sources to decolorize remazol golden yellow dye using individual bacterial cultures and to find out optimal co-substrate to enhance the efficacy of decolorization process.

Materials and Methods

Isolation and identification of microorganisms : Bacterial isolates were isolated from textile industry effluent contaminated soil. Morphologically distinct bacterial isolates were screened for decolorization in Luria bertani broth (g l^{-1}) containing casein enzymic hydrolysate 10 g, yeast extract 5 g and sodium chloride 10 g along with remazol golden yellow (RNL) at 100 mg l^{-1} concentration in pH 7 under static conditions for 48 hrs. Further, the bacterial isolates were characterized based on the standard biochemical approach.

Effect of various carbon and nitrogen sources on dye degradation : Effect of various carbon sources such as glucose, sucrose, lactose, starch, fructose and nitrogen sources beef extract, yeast extract, peptone, ammonium nitrate, ammonium sulphate and urea were utilized to find out their efficiency to degrade remazol golden yellow using isolated bacterial strains. About 100 ml of Bushnell hass broth containing (g l^{-1}): magnesium sulphate 0.2 g, dipotassium hydrogen phosphate 1.0 g, calcium chloride 0.02 g, ferric chloride 0.05g and ammonium nitrate 1.0g amended with 1% carbon and nitrogen source were prepared respectively in 250 ml flask and sterilized at 121°C. To it, filter sterilized remazol golden yellow dye at 200 mg l^{-1} was added. The, 5% of each 24 hr old bacterial inoculums was seeded into respective carbon and nitrogen sources and incubated under static condition for 12 days.

Analysis of decolorization : Colorimetric analysis was employed to monitor the dye decolorization ability of six bacterial isolates in decolorized broth culture (Sani and Banerjee, 1999). About 5 ml of decolorized broths were withdrawn aseptically at usual interval of 24 hrs and centrifuged with 3000 rpm for 15 min. The culture filtrate was used to analyze the percentage of decolorization at 595 nm for remazol golden yellow. Percentage decolorization = [(Absorbance of uninoculated broth- Absorbance of residual broth) /Absorbance of uninoculated broth] x 100.

Growth analysis : In relation to decolorization growth of the bacterial strains was monitored by a colorimeter. The cell pellet obtained from centrifugation (3000 rpm for 15 min) of 5 ml decolorized broth was resuspended in 5 ml distilled water and its absorbance was noticed at 660 nm.

Results and Discussion

The pH of the samples was found to be within range of pH 8-9. Total heterotrophic bacterial population in soil was 62 x 10^6 CFU g^{-1}, 2 x 10^4 CFU ml^{-1} in the effluent. Majority of bacterial genera in the samples belonged to *Bacillus* sp., *Enterobactericeae, Lactobacillus* sp., *Pseudomonas* sp., *Alcaligenes* sp., *Aeromonas* sp., *Staphylococcus* sp., and *Micrococcus* sp. The result of the present study clearly demonstrates that the soil samples from the dye contaminated site had high bacterial population (62 x 10^6 CFU g^{-1}) as compared to sludge (9 x 10^4 CFU g^{-1}) and textile effluent samples (12 x 10^6 CFU ml^{-1}). It has been well proven that the isolates from dye contaminated soil naturally resistant to withstand the toxic chemicals and salts (Khehra *et al.,* 2005).

Screening of dye decolorization for bacterial strains were carried out from a series of isolation and identification experiments in Luria bertani medium using remazol golden yellow dye at 100 mg l^{-1} concentration. At the end of 48 hrs of incubation period, *Staphylococcus* sp. (ES-37), *Bacillus* sp. (ESL-52), *Bacillus* sp. (TSL-9), *Micrococcus* sp. (TSL-7), *Lactobacillus* sp. (TS-5) and *Pseudomonas* sp. (M-1) exhibited maximum decolorization at pH 7, under static condition. All the six bacterial isolates exhibited maximum decolorization (68-85%) only under static condition (Table 1). Dye concentration used in the present study was 200 mg l^{-1}, since textile industry effluent contained dye in the range of 0.1-0.2 g l^{-1}. Previous studies have reported that low concentration produced higher color removal and decolorization percentage decreased at high dye concentration because of the toxicity of dyes to microorganisms (Gomare *et al.,* 2009). To establish the effect of carbon and nitrogen source by adapting the experimental design of one factor at a time by keeping other factors constant was a

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Table 1: Screening of bacterial isolates for dye decolorization (Remazol golden yellow)

Tentative Genus	Optical Density Values		Decolourization
	Initial	Final	%
Staphylococcus sp. (ES-37)	0.75	0.24	68
Bacillus sp. (ESL-52)	0.75	0.14	81
Bacillus sp., (TSL-9)	0.75	0.14	80
Micrococcus sp. (TSL-7)	0.75	0.16	78
Lactobacillus sp. (TS-5)	0.75	0.14	80
Pseudomonas sp. (M-1)	0.75	0.11	85

valuable method used to investigate the role of single factor in an occurrence.

Bacterial strains ESL-52 (84.21%), TSL-7 (58.55%) and TS-5 (53.94%) exhibited maximum decolorization by utilizing starch at 1% (w/v), as a significant substrate, to enhance the decolorization process. Strain ES-37 (84.16%), M-1 (56.41%) and TSL-9 (60.25%) showed maximum decolorization activity in the decolorized medium amended with 1% lactose acted as a notable co-substrate (Fig.1). The effect of carbon sources on dye decolorization clearly indicate that color removal was not dependent on growth. However, dye removal was more significant between growth of bacterial isolates and selective carbon sources.

Results for effect of carbon source on remazol golden yellow decolorization with bacteria showed starch and lactose as the main source for removal of dye. Few researches were only successful in obtaining microorganism capable of utilizing azo dyes as their sole carbon source. Azo dyes are deficient in carbon source therefore the color removal was feasible only with co - metabolite condition (Chang *et al.,* 2004). In contrast, addition of carbon source, instead of increasing decolorization, reduces the rate possibly due to assimilation of added carbon source and over using dye as a carbon source (Saratale *et al.,* 2009). *Pseudomonas* sp., M1 decolorized dye in the presence of lactose was 56.41%, whereas fructose, starch and lactose decolorized the dye by 91 to 93% by *Pseudomonas* sp. (Jyoti *et al.,* 2010). *Bacillus laterosporus* decolorized navy blue-3G in the synthetic medium, containing glucose and starch. This was closely related in the present study to *Bacillus* sp., ESL-52 strain but differed with *Bacillus* sp., TSL-9 (Gomare *et al.,* 2009). TSL-9, ES-37 and M-1 use lactose as a carbon source for the effectual decolorizarion. They also used 5 g l^{-1} lactose as the most needed carbon source for decolorization of everzol red RBN (Junnarkar *et al.,* 2006). Recent reports found decolorization by starch was slow compared to glucose due to low activity of the glycoside hydrolase enzyme, but the mixture of starch and glucose attained good yield of color removal (Boonyakamol *et al.,* 2009). Khehra *et al.* (2005) showed that glucose act as a co-substrate to increase the decolorization of RV5R with an increase in the concentration of dye up to 2.5 g l^{-1}. This was mainly due to the production of NADH, FADH from glucose metabolism.

All the strains showed evidence of maximum color removal on 7th day of incubation. The presence of yeast extract and beef extract increased the color removal while other nitrogen sources like peptone, urea, ammonium sulphate and ammonium nitrate showed a poor decolorization rate. Maximum decolorization of remazol golden yellow dye was 82.35% by the strain ESL-52 followed by 80.23, 52.94 and 53.52% using ES-37, TS-5 and M-1 strains, respectively, in the presence of 1 % (w/v) yeast extract as a nitrogen source. Similarly, the bacterial strains TSL-9 and TSL-7 stimulated significant decolorization about 53.29 and 77.24%, respectively in the medium incorporated with 1% beef extract (Fig.2).

Bacterial growth was best in the medium amended with organic nitrogen sources (yeast extract, beef extract and peptone), while inorganic nitrogen sources (ammonium nitrate, ammonium sulphate and urea) did not influence the growth of the bacterial strains during the dye decolorization process.

Effect of nitrogen source suggested that addition of organic nitrogen source yeast extract and beef extract favored better decolorization. Organic nitrogen source yeast extract was considered as an essential media supplement to regenerate NADH in the medium, which act as an electron donor for reduction of dyes by bacteria (Hu, 1994). Reports have also shown that decolorization of Ranocid fast blue dye was best in the media containing yeast extract (Mathew and Madamwar, 2004). The present study signifies that peptone and other inorganic nitrogen sources failed to enhance the color removal by bacterial species is in agreement with the previous study of Gomare *et al.* (2009). The color removal percentage, of a wide range of dyes, increased with the addition of yeast extract (Bayoumi *et al.,* 2010). However, literature survey supports that yeast extract as a best co inducer for bacterial isolates (Chen *et al.,* 2003; Moosvi *et al.,* 2005).

Bacterial strains decolorize the dye concentration of 200 mg l^{-1} showed decrease normally after 7th day. Similar results were observed for decolorization of reactive green 19A by *Micrococcus glutamicus* (Saratale *et al.,* 2009). Decrease in decolorization of remazol golden yellow was due to the degraded product accumulated together produced a new color in broth and nutrient depletion affects the metabolism of microorganisms. Other cause includes that toxicity of the dye to bacterial cells and inadequate biomass concentration of dye (Jadhav *et al.,* 2008). Further the chemical structure of the dyes also affected the rate of decolorization. An increase in dye concentration also made the medium acidic. Dyes with low molecular weight and simple structure were degraded easily in bulk quantities but in case of dyes, with complex structure make, it was hard to break the ring led to the time consuming process (Elisangela *et al.,* 2009). In the presence of glucose and yeast extract, as co-substrate, a

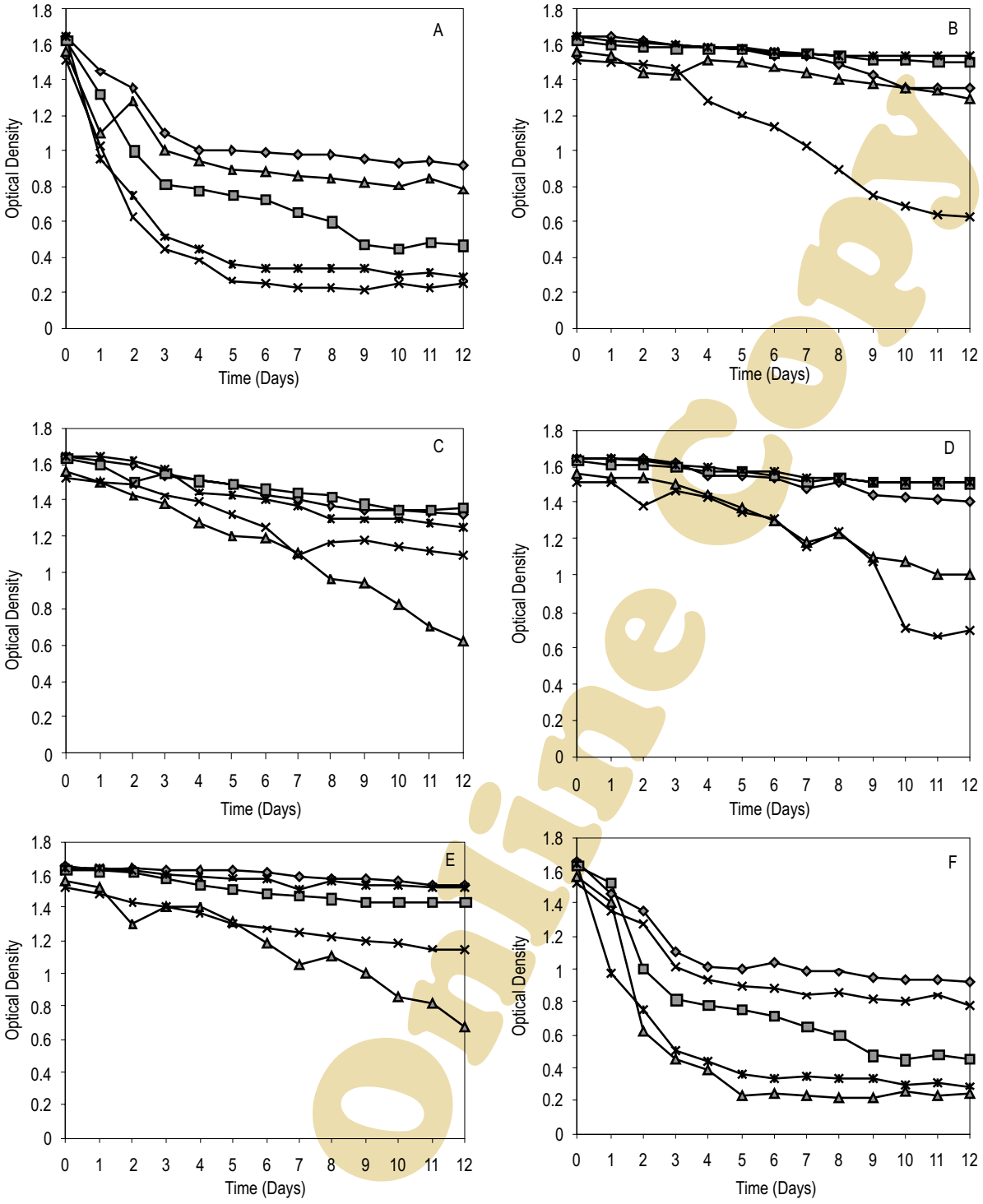

Fig. 1 : Effect of carbon sources on the decolorization of Remazol golden yellow enriched with bacterial strains. A. ESL-52, B. TSL-9, C. TSL-7, D. TS-5, E. M-1 and F. ES-37. ♦ Glucose, ■ Sucrose, ▲ Lactose, × Starch and ✳ Fructose.

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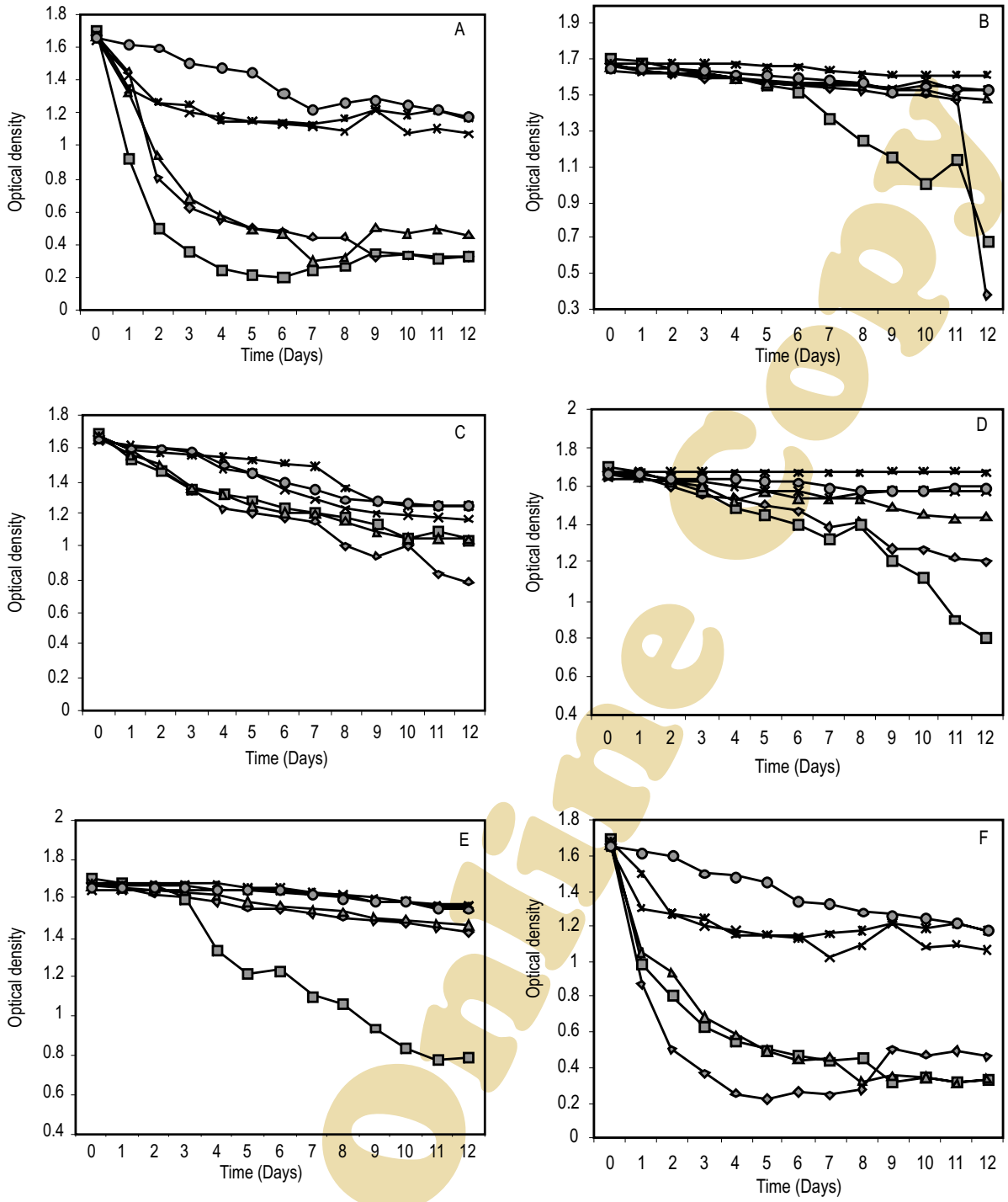

Fig. 2 : Effect of nitrogenous sources on the decolorization of Remazol golden yellow enriched with bacterial strains. A. ESL-52, B. TSL-9, C. TSL-7, D. TS-5, E. M-1 and F. ES-37. ♦ Beef Extract, ■ Yeast Extract, ▲ Peptone, ✕ Ammonium Nitrate, ✱ Ammonium Sulphate and ● Urea

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number of cultures utilizing RV5R were isolated from soil samples but a few strains failed to decolorize by successive transfer on second and third time (Nigam *et al.,* 1996).

The present study revealed that addition of 1% starch and yeast extract in the medium improved the color removal. This was inconsistent with earlier reports of Jang *et al.* (2007) where 1% glucose increased the decolorization process, but in case of nitrogen source did not affect the color removal effectiveness of textile dye. It was found that 0.1% of the starch as carbon source showed faster removal of Ranocid fast blue dye (Chen *et al.,* 2003). The optical density and percentage of decolorization value of all the substrates implies the need, intake and applicability of each co-substrate for dye decolorization.

Generally, the percentage decolorization was best in co-substrate amended media. This result was correlated with Moosvi *et al.* (2005) in which starch and yeast extract in the medium enhanced biodegradation process. In the absence of supplementary carbon and nitrogen source, the bacterial culture was unable to decolorize dye representing the essentiality of co-substrate for growth and decolorization (Nigam *et al.,* 1996). The presence of carbon and nitrogen either showed inhibitory or stimulatory effect on decolorization of direct orange 39, resulting the variation in the time required for decolorization along with percentage decolorization (Jyoti *et al.,* 2010).

The efficiency of Remazol golden yellow decolorization was slow. The results clearly indicated that the individual and co-substrate had different impact on the utilization of dye, and inherited the growth of bacterial isolates in the respective fermentation medium. Decolorization rate decreased normally, after a week of incubation due to accumulation of degraded metabolites. It was concluded that the culture condition was not well optimized with the rest of parameters, hence it is evident to carry out further optimization to produce a wide range of complete dye removal.

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References

Aleboyeh, A., Y. Moussa and H. Aleboyeh: The effect of operational parameters on UV/H_2O_2 decolorization of Acid Blue 74. *Dyes Pigments,* **66**, 129-134 (2005).

Bayoumi, R.A., S.M. Musa, A.S. Bahobil, S.S. Louboudy and T.A. El-Sakawey: Biodecolourizarion and biodegradation of azo dyes by some bacterial isolates. *J. Appl. Environ. Biol. Sci.,* **1**, 1-25 (2010).

Bi, X., P. Wang, C. Jiao and H. Cao: Degradation of remazol golden yellow dye wastewater in microwave enhanced ClO2 catalytic oxidation process. *J. Hazard. Mater.,* **168,** 895–900 (2009).

Boonyakamol, A., T. Imai, P. Chairattanamankorn, T. Higuchi, M. Sekine and M. Ukita: Reactive blue 4 decolorization under mesophilic and thermophilic anaerobic treatments. *Appl. Biochem. Biotechnol.,* **152**, 405-417 (2009).

Chang, J.S., B.Y. Chen and Y.S. Lin: Stimulation of bacterial decolorization of azo dyes by extracellular metabolites of *Escherichia coli* strain NO3. *Bioresour. Technol.,* **91**, 243-248 (2004).

Chen, K.C., J.Y. Wu, D.J. Liou and S.C. John Hwang: Decolorization of the textile dyes by newly isolated bacterial strains. *J. Biotechnol.,* **101**, 57-68 (2003).

Chen, Y., S. Yang, K. Wang and L. Lou: Role of primary active species and TiO_2 surface characteristic in UV-illuminated photodegradation of Acid Orange 7. *J. Photochem. Photobiol. A.,* **172**, 47-54 (2005).

Elisangela, F., Z. Andrea, D.G. Fabio, R.M. Cristiano, D.L. Regina and C.P. Artur: Biodegeradation of textile azo dyes by facultative *Staphylococcus* strain VN-11 using a sequential micro aerophilic/ aerobic process. *Int. Biodeterior. Biodegrd.,* **63**, 280-288 (2009).

Gomare, S.S., S.D. Kalme and S.P. Govindwar: Biodegradation of navy blue-3G by *Brevibacillus laterosporus* MTCC 2298. *Acta. Chim. Slav.,* **56**, 789-796 (2009).

Hu, T.: Decolorization of reactive azo dyes by transformation with *Pseudomonas luteola. Bioresour. Technol.,* **49**, 507-516 (1994).

Jadhav, U.U., V.V. Dawkar, G.S. Ghodake and S.P. Govindwar: Biodegradation of direct red 5B, a textile dye by newly isolated *Comamonas* sp. UVS. *J. Hazard. Mater.,* **158**, 507-516 (2008).

Jang, M.S., B.G. Jung, N.C. Sung and Y.C. Lee: Decolorization of textile plant effluent by *Citrobacter* sp. strain KCTC 18061P. *J. Gen. Appl. Microbiol.,* **53**, 339-343 (2007).

Jin, X.C., G.Q. Liu, Z.H. Xu and W.Y. Tao: Decolorization of a dye industry effluent by *Aspergillus fumigatus XC6*. *Appl. Microbiol. Biotechnol.*, **74**, 239-43 (2006).

Junnarkar, N., D. Srinivas Murty, N.S. Bhatt and D. Madamwar: Decolorization of diazo dye direct red 81 by a novel bacterium consortium. *W. J. Microbio. Biotechnol.,* **22**, 163-168 (2006).

Jyoti, P.J., S.P. Swapnil, S.D. Rhishikesh and B.J. Shekhar: Rapid biodegradation and decolorization of direct orange 39 (Orange TGLL) by an isolated bacterium *Pseudomonas aeruginosa* strain BCH. *Biodegradation,* **21**, 453-463 (2010).

Khehra, M.S., H.S. Saini, D.K. Sharma, B.S. Chadha and S.S. Chimni: Comparative studies on potential of consortium and constituent pure bacterial isolates to decolorize azo dyes. *Water Res.,* **39**, 5135–5141 (2005).

Kuhad, R.C., N. Sood, K.K. Tripathi, A. Singh and O.P. Ward: Development in microbial methods for the treatment of dye effluents. *Adv. Appl. Microbiol.,* **56**, 185-213 (2004).

Mathew, S. and D. Madamwar: Decolorization of Ranocid fast blue dye by bacterial consortium SV5. *Appl. Biochem. Biotechnol.,* **118**, 371-381 (2004).

Moosvi, S., H. Keharia and D. Madamawar: Decolorization of textile dye reactive violet 5 by a newly isolated bacterial consortium RVM 11.1. *W. J. Microbio. Biotechnol.,* **21,** 667-672 (2005).

Nigam, P., I.M. Banat and R. Marchant: Decolorization of effluent from the textile industry by a microbial consortium. *Biotechnol. Lett.,* **18**, 117-20 (1996).

Ou, Y., J.D. Lin, H.M. Zou and D.W. Liao: Effects of surface modification

of TiO_2 with ascorbic acid on photocatalytic decolorization of an azo dye reactions and mechanisms. *J. Mol. Cat. A.,* **241**, 59–64 (2005).

Peternel, I.T., N. Koprivanac, A. M. Bozic and H. M. Kusic: Comparative study of UV/TiO_2, UV/ZnO and photo-Fenton processes for the organic reactive dye degradation in aqueous solution. *J. Hazard. Mater.,* **148**, 477-484 (2007).

Robinson, T., G. McMullan, R. Marchant and P. Nigam: Remediation of dyes in textile effluent: A critical review on current treatment technologies with a proposed alternative. *Bioresour. Technol.,* **77**, 247-255 (2001).

Sani, R.K. and U.C. Banerjee: Screening for organisms applicable to the decolorization of triphenylaniline dyes and optimization of biotransformation conditions in stirred tank reactor. *Indian. J. Environ. Ecoplan*., **2**,1-9 (1999).

Saranaik, S. and P. Kanekae: Biodegradation of methyl violet by *Pseudomons menolocrin* MC MB-402. *Appl. Microbiol. I.,* **52**, 251-254 (1999).

Saratale, R.G., G.D. Saratale, J.S. Chang and S.P. Govindwar: Ecofriendly degradation of sulfonated diazo dye C.I. Reactive green 19A using *Micrococcus glutamicus* NCIM-2168. *Bioresour. Technol.,* **100**, 3897-3905 (2009).

Srinivasan, S.V. and D.V. Murthy: Statistical optimization for decolorization of textile dyes using *Trametes versicolor. J. Hazard. Mater.,* **165**, 909–914 (2009).

Syed, M.A., H.K. Sim, A. Khalid and M.Y. Shukor: A simple method to screen for azo-dye-degrading bacteria. *J. Environ. Biol.*, **30**, 89-92 (2009).

Zhang, Z. and H. Zheng: Optimization for decolorization of azo dye acid green 20 by ultrasound and H_2O_2 using response surface methodology. *J. Hazard. Mater.,* **172**, 1388–1393 (2009).

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