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Bioremediation of textile effluent using bacterial consortium obtained from industrially polluted site

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ABSTRACT

The inappropriate and unselective dumping of textile effluent in natural waters and land is creating serious complications and it has also antagonistic effects on various crops. The effect of various combinations of selected isolates on textile effluent decolorization was assessed, to develop an effective consortium. The consortium of isolate SPR_{28} and SPR_{42} (1:1) displayed maximal decolorization of 42.5% after 84 h of incubation. The consortium of isolate, SPR_{28} and SPR_{48} (1:1) displayed maximum decolorization of 39.8% after 96 h of incubation. Molecular characterization of the 16S rRNA sequences showed similarity of isolate SPR_{42} with *Bacillus subtilis* and isolate SPR_{48} to*Bacillus cereus.* toxicity test of bioremediated effluent was performed at 100% conc. of effluent. Untreated effluent showed 30.3% seed germination and 3.03 germination speed. On the other hand, seed exposed with bioremediated effluent lead to% germination and germination speed of 98.7% and 10.8, respectively, for consortium of SPR_{42} and SPR_{48}. However, seed treated with *P. putida* bioremediated effluent showed 70.9% germination with a germination speed of 5.16. Microbial decolourization holds potential and would be used to advance a low cost, biodegradable microbial technology for the removal of textile dyes/effluent.

Key words: *Textile effluent, Bioremediation, Vigna radiata, Microbial consortia*

Introduction

Textile companies have been grouped in the class of the utmost polluting manufacturing units by various government agencies. India has a huge system of textile trades of variable size that are scattered throughout the country. The increasing population and advanced adoption of an industrial-based life style has certainly led to an enlarged anthropogenic impact on the atmosphere. In textile processing, chances exist for the release into the ecosystem of possibly hazardous substances at different stages of the operation and these pollutants impact undesirably on the environment (Dwivedi and Tomar, 2018). The inappropriate and unselective discharge of textile effluent in natural water and land is posturing serious difficulties and it has also adverse effects on different crops (Rathode and Pathk, 2014). Textile waste reduces seed growth and germination growth of all vegetable and this effect is more distinct at the high amount of textile effluent (Rehman *et al.,* 2009). The incidence of even very low concentration of dyes in effluent is extremely visible and objectionable (Nigam *et al.,* 2000). Thus, the decolourization of textile wastewater has been a foremost environment apprehension since a long time. Diverse methods are used for the decolourization of textile wastewater; mechanical, chemical, physico-chemical and biological or in combination (Schulze-Rettmer, 1998; Parac-

Osterman *et al.,* 2007; Vikrant *et al*., 2018).

Bioremediation is attractive significant approach, as of its efficacy and environment responsive nature (Robinson *et al.,* 2001). Many microorganisms can decolourize the azo dyes under laboratory and field conditions (Sani and Banerjee, 1999; Moosvi *et al.,* 2005) and fungi (Balanand Monteneiro, 2001; Verma and Madamwar, 2005). Environmental microbiology trusts upon the pollutants degrading potential if naturally occurring microorganisms in which bacteria play central role. Bioremediation in alkaline environment (due to high pH of effluent) certainly needs the claim of microorganisms, which can grow under exacting conditions. Subsequently augmented bacteria may have some toxic effects on the atmosphere, applying or stimulating the indigenous micro-flora is chosen if possible. Moreover, the bacterial consortia aremore operative than individual cultures in bioremediation because of their broaderenzymatic potential (Moosvi *et al.,*2007; Mahmood *et al*., 2015). So, the current study was focused on the developing effective microbial consortia from aerobic textile bacteria isolated from textile waste contaminated soil of an industrial estate. The effect of the non-treated and bio-treated effluent was investigated on the germination of *Vigna radiata*.

Methodology

Sample collection

Textile effluent was obtained from Sewage treatment unit of a local textile manufacturer. The main site was effluent treatment plant (ETP), where effluent was stored for chemical treatment before disposal. The samples were obtained in pre-sterilized plastic bottles. The colour of liquid waste was dark reddish brown. The different soil samples (affected) were obtained aseptically from many sites of industry as Effluent treatment plant (ETP), fresh sludge disposal site and oldsludge dumping site.

Isolation and screening of textile effluent decolourizing bacteria

All the chemicals and media composition were procured from Hi Media, Mumbai. The textile Azo dyes: Golden Yellow 3RL (G.Y. 3RL), Yellow HEGR (Y. HEGR), Red HE7B (R. HE7B), Congo Red (CR) and Triphenylmethane dye - Malachite Green (MG) were taken from textile industry (Shivalik Polymer Ltd.) Faridabad, India. The standard type culture, *Pseudomonas putida* (MTCC 2445), was used during the present study.

The isolation of textile dye degrading microbe from soil samples was performed by serial dilution using nutrient medium containing different dyes (G.Y 3RL,Y. HEGR,R. HE7B, CR and MG) at concentration of 10 mgL^{-1}. The incubation was done at 37 °C for 5 days. Pure colonies of bacteria based on their morphology were subculture and maintained at 4 °C. The bacterial isolates were further screened for the decolourization of textile effluent using modified mineral broth containing various concentration of textile effluent ranging (25%, 50%, 75% and 100% (v/v)) and incubation at 37 °C for 5 days. The decolourization was studied at regular interval of 12 h. The amount of decolourization was recorded spectrophotometrically (*i.e.* G.Y 3RL at 400 nm, Y. HEGR – 400 nm, R. HE7B – 550 nm, CR – 530 nm, MG – 620 nm and Textile effluent – 555 nm). Decolourization efficacy was stated in terms of % decolourization (Sani and Banerjee, 1999).

$$\text{Decolourization (\%)} = \frac{\text{Initial absorbance - Final absorbance}}{\text{Initial absorbance}} \times 100$$

Combination of efficient bacterial consortium

For the formation of potential consortium for bioremediation of textile waste effluent, the various combinations of isolates, which were individually showing maximum decolourization of textile effluent, were inoculated to 100 mL effluent (pH-7.0) in the ratio of 1:1. The inoculated effluents were incubated at 37 ºC for 4 days. About 4 mL sample was taken out after every 12 hrs intervals and centrifuged at 9,000 rpm for 20 min, the cell free extracts were withdrawn and used for decolourization assay spectrophotometrically.

Effect of inoculums ratio of selected consortium

To study the effect of various inoculums ratios of isolates in selected consortium on textile effluent decolourization, isolates were inoculated in textile effluent in varying ratio – 1:1, 1:2, 2:1. The inoculated effluents were incubated at temperature 37 ºC for 4 days. Samples were taken out every 12 hrs intervals and decolourization was studied.

Optimization of decolourization process parameters by selected consortium

The effect of temperature (15 ºC, 25 ºC, 37 ºC, 45 ºC

and 55 ºC), pH (5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5 and 10.0) and aeration (shaking at 200 rpm and non-shaking) on the ability of selected consortium to decolourize textile effluent was studied. Textile effluent (100%) was inoculated with selected consortium and incubated for 4 days. The decolourization activity was calculated as earlier.

Identification and characterization of isolates

For molecular phylogenic determination, the genomic DNA was extracted using modified as demonstrated by Charles and Nester (1993). The rDNA sequence was identified using different primer 41f and 1488r (Maatallah *et al*., 2002). The amplified genomic material was purified on agarose gel with marker (Genei) and the desired band was purified and sequenced commercially. The sequence was presented to GenBank and accession ID were allocated.

Phylogenetic Tree Construction

Molecular identification of consortium was resolute by BLAST alignment, gene sequences were sorted and aligned using Clustal W(Saitou and Nie, 1987; Felsenstein, 1985).

Toxicity assessment

Toxicity of raw and remediated textile effluent was tested on the germination of *V. radiate* seeds. Seed germination studies were carried out using the Petri dish method (Ranga *et al*., 2015). The seeds of *V. radiate* were sterilized with 0.1% mercurous chloride and then extra mercurous chloride was removed using double distilled water. The result of various concentrations of non-treated effluent ranging (25-100%, v/v) textile waste effluent was recorded on seeds. Plant samples were dried in an oven for overnight at 45 °C and the dry weight was recorded.

$$\text{(a) \% germination} = \frac{\text{No. of seeds germinated}}{\text{Total no. of seeds sown}} \times 100$$

$$\text{(b) Germination speed} = \frac{\text{\% germination}}{\text{Days of completion of germination}}$$

(c) Vigour index = % germination x shoot length

$$\text{(d) Peak value} = \frac{\text{Peak germination \%}}{\text{Peak count day}}$$

$$\text{(e) Emergence index} = \frac{\text{No. of seeds germinated on particular day}}{\text{Days of emergence}}$$

Results and Discussion

Isolation and Screening of bacteria

About 58 bacterial isolates were obtained from the samples collected and were named as SPR_1 to SPR_{58} for further studies. The isolates $SPR_{28,}$ SPR_{42} and SPR_{48} showed very good decolourization of dyes G.Y. 3RL, Y. HEGR, R. HE7B (Reactive Red 141) and CR (Supplementary Table 1). Whereas, in case of MG, the isolates $SPR_{28,}$ $SPR_{42,}$ SPR_{48} and SPR_{53} showed very good decolourization (Table 1). The dye concentration of 10-200 mgL^{-1} was used for study because textile effluent also contains dyes in same concentration (Padmavathy *et al.*, 2003; Chang *et. al.*, 2001). The isolates SPR_8, SPR_{11}, SPR_{12}, SPR_{28}, SPR_{34}, SPR_{42}, SPR_{46}, SPR_{48}, SPR_{51}, and SPR_{57} showed good decolourization of textile effluents (Supplementary Table 1). Earlier studiesalso showisolation of dye decolourizing bacteria from soil samples collected from dye manufacturing industries and efflu-

Table 1. Germination of seeds by untreated and bioremediated effluent.

Sr. No.	Parameter(s)	Untreated Effluent	Bio-Treated textile waste	
			SPR_{42} + SPR_{48}	*P. putida*
1	Germination %	30.3± 0.67	98.7± 0.71	70.9± 0.61
2	Rate	3.03± 0.51	10.8± 0.44	5.16± 0.44
3.	Emergence Index	2.0± 0.40	9.8± 0.46	7.10± 0.51
4	Peak Value	3.03± 0.35	10.8± 0.52	5.16± 0.29
5	Vigor Index	0.582± 0.42	595.7± 0.56	274.5± 0.46
6	Shoot parameters	0.067± 0.32	6.21± 0.45	3.110± 0.44
7	Root parameters	0.160± 0.54	4.08± 0.43	3.13± 0.45
8	Wet Root Weight	2.5± 0.38	123.8± 0.59	40.50± 0.50
9	Wet shoot weight	18.0± 0.47	356± 0.70	76.43± 0.56
10	Dry Root weight	0.503± 0.31	14.8± 0.55	1.94± 0.33
11	Dry shoot weight	5.01± 0.44	17.3± 0.51	7.84± 0.46

ent treatment plant sites (Sani and Banerjee, 1999; Dawkar *et al.*, 2008; Dave and Dave, 2009).

The secondary screening of the isolates on different concentrations of textile effluent showed that all the isolates were explore to decolourize the textile effluent at concentration of 25% (v/v) and the decolourization was increased variably till 96 h of incubation (Figure 1A). The reference isolate *P. putida* showed maximum decolourization of 44.8% after 96 h of incubation. The ten isolates (SPR8, SPR11, SPR12, SPR28, SPR34, SPR42, SPR46, SPR48, SPR_{51} and SPR_{57}) decolourizing the textile effluent efficiently were selected for further studies. At 50% (v/v) concentration of textile effluent, isolates SPR_{28}, SPR_{42}, $SPR_{48}$and *P. putida* showed maximum decolourization of 68.3%, 72.4%, 66.2% and 39.5% respectively after 96 h of incubation (Figure 1B). Similarly, at 75% and 100% (v/v) concentration of textile effluent, maximum decolourization was observed after 96 h of incubation in isolates SPR_{28}, SPR_{42}, SPR_{48}(Figure 1C & 1D). However at 100% concentration, the decolourization by isolates, SPR_{28}, SPR_{42}, SPR_{48},was reduced to 29.3%, 39.1% and 26.6%, respectively (Figure 1D). Previous studies also suggest that effluent adapted bacterial strains were better candidates for decolourizing the textile effluent than the non-adapted species (Leena and Selva Raj, 2008; Mahmood *et al.*, 2015).

Development of consortium for bioremediation

The effect of various combinations of selected isolates on textile effluent decolourization was assessed, to develop an effective consortium. The consortium of isolate SPR_{28} and SPR_{42} (1:1) showed maximum decolourization of 42.5% after 84 h of incubation (Figure 2A). The consortium of isolate, SPR_{28} and SPR_{48} (1:1) showed maximum decolourization of 39.8% after 96 h of incubation (Figure 2A). While the consortium of isolates SPR_{42} and SPR_{48} (1:1) showed 51.2% decolourization after 72 h of incubation (Figure 2A). A consortium of isolates SPR_{28}, SPR_{42}, and SPR_{48} in equal amount

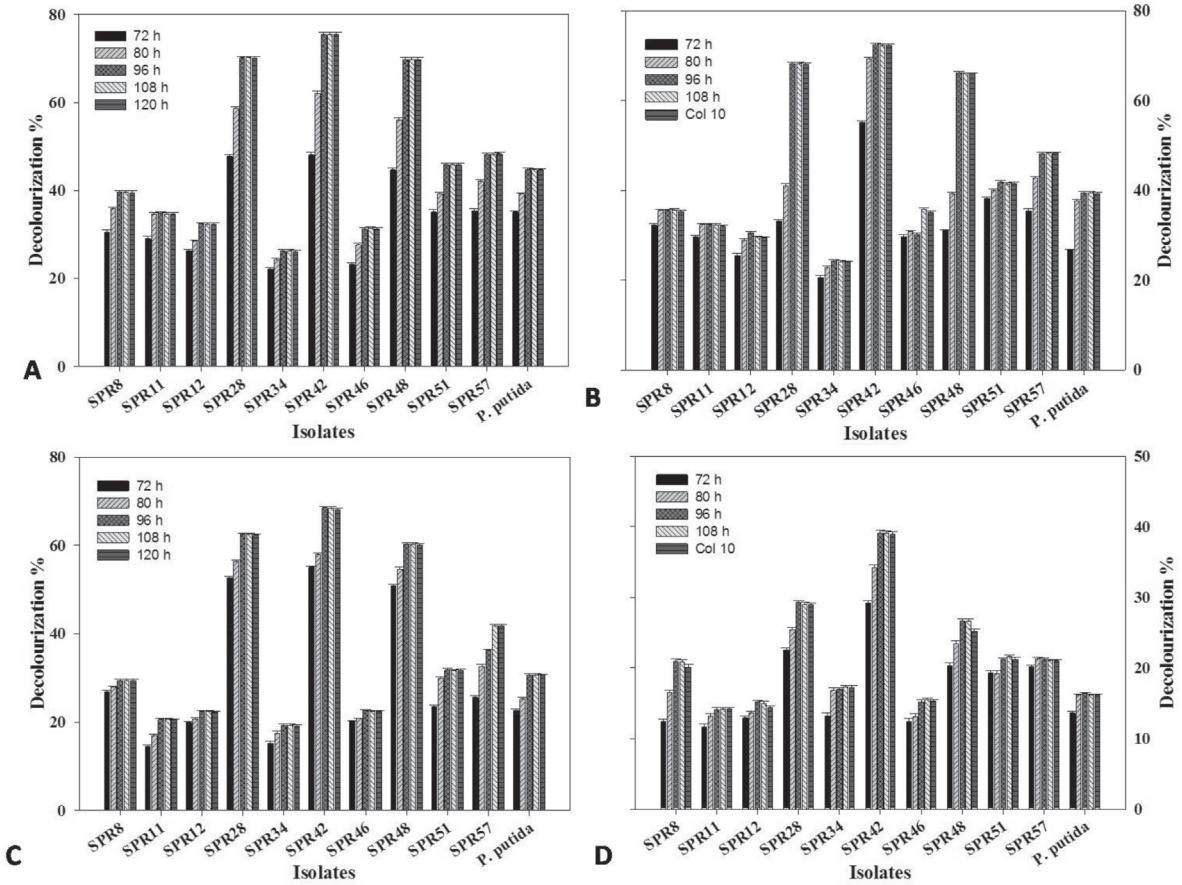

Fig. 1. Secondary screening of isolates for decolourizing textile effluent at different concentration: A. 25%; B. 50%; C. 75% and D. 100%.

showed maximum decolourization of 18.4% after 96 h of incubation (Figure 2A).

Previous studies also show that bacterial consortium is more efficient in decolourizing textile dyes compared to individual isolates. Moosvi *et al.* (2007) selected a microbial consortium (*Paenibacillus polymyxa*, *M. luteus* and *Micrococcus* sp.) showing maximum decolourization of textile dyes (Reactive Violet 5R) and textile effluent, however, separate isolates did not show decolourization even on prolonged incubation.

Effect of inoculum ratio of selected consortium

The consortium of isolates SPR_{42} and SPR_{48} in ratio of 1:1 showed maximum decolourization. The change in ratio of the isolates SPR_{42} and SPR_{48} to 1:2 showed decrease in decolourization efficiency with maximum decolourization of 37.8% after 72 h of incubation (Figure 2B). However when these two isolate SPR_{42} and SPR_{48}were added in the ratio of 2:1 in the consortium, the maximum decolourization was 28.2% after in 72 h of incubation (Figure 2B). Thus, the 1:1 ratio of isolates was used for further studies.

Bafana *et al*. (2007) isolated a group of *Cardiobacterium hominis* and *Pseudomonas stutzeri* from industrial waste of a textile industry based on its potential to decolourize azo dyes. Kumar *et al*. (2007) observed decolourization of Direct Blue-15 by a mixed bacterial consortium of isolates *Sphingomonas* sp. EBD, *B. subtilis*, *B. thuringiensis*, *Enterobacter cancerogenus* and *Alcaligenes faecalis*. Tony *et al.* (2009) developed consortium SKB-II consisted of five diverse bacterial types *B. vallismortis*, *B. pumilus*, *B. cereus*, *B. subtilis* and *B.megaterium* which were most efficient in decolourizing distinct as well as combination of dyes and textile effluent also.

Optimization of decolourization process parameters by selected consortium

The outcome of different incubation temperature on the dye detoxification efficiency of the consortium of isolate $SP_{R4}2$ and $SP_{R4}8$ (1:1) was studied. The maximum decolourization of 51.8% was observed at 37 °C after 72 h of incubation (Figure 3A). The consortium of isolate SPR_{42} and SPR_{48} showed no decolourization at 15 ºC and 55 ºC. At 25 ºC optimal activity of 30.0% was detected after 72 h of incubation (Figure 3A). At 45 ºC the consortium showed maximum activity of 40.9% after 72 h of incubation (Figure 3A).

The effect of various pH on the decolourization efficiency of the consortium was also studied. The consortium showed very good decolourization of 40.1% within 12 hrs of incubation at pH 8.5 with maximum decolourization of 72.2% after 72 h of incubation (Figure 3B). However, no decolourization was observed at pH 5.0, 5.5, 6.0 and 10.0. The effect of aeration on decolourization efficiency of consortium showed enhanced decolourization in static conditions as compared to shaking conditions (Figure 3C). In static conditions maximum decolourization of 72.8% was observed after 72 h of incubation. However in shaking conditions, consortium showed maximum decolourization of 61.6% after 72 h of incubation. Earlier, Senan and Abraham (2004) developed a microbial consortium

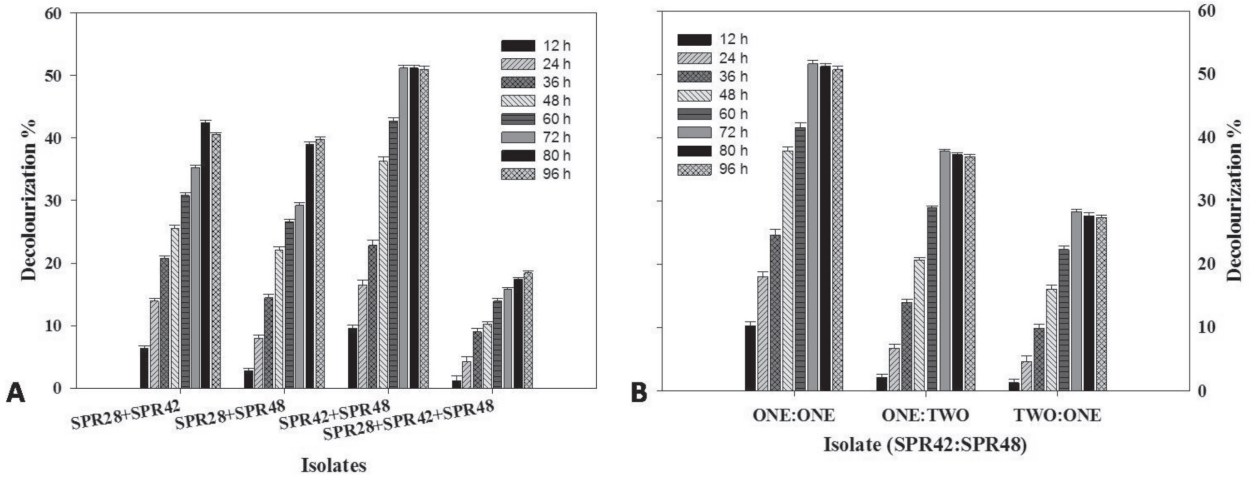

Fig. 2A. Development of consortium for textile effluent bioremediation.
B. Effect of inoculum ratio of selected consortium.

composed of isolated strains and a strain of *Pseudomonas putida* (MTCC1194) for degradation of a combination of azo dyes at pH (9-10.5) and salinity (0.9-3.68 gL^{-1}) at room temperature (28 ± 2 °C). It showed complete decolourization of textile effluent and Reactive Orange 16 (100 mgL^{-1}) within 48 hrs at pH 7 and 30 °C. Jadhav *et al.* (2010) obtained a consortium consisting three bacterial sp. (*Pseudomonas sp*.) from dye contaminated sites capable of decolourizing dye quicker than the distinct bacteria under non shaking conditions.

Molecular identification and Phylogenetic analysis

The PCR amplification of 16S rRNA gene of isolates SPR_{42} and SPR_{48} with 16S rRNA primers gave amplification of about 1500 bp. The 16S rRNA gene sequences of the bacterial isolates $SPR_{42}$and SPR_{48}were presented to NCBI Genbank under ID GQ872346 and HQ316117 respectively. Phylogenetic analysis of the 16S rRNA sequences showed similarity of isolate $SPR_{42}$with *Bacillus subtilis* and isolate SPR_{48} to *Bacillus cereus* (Figure 4) which has earlier been used for textile dye decolourization (Dawkar *et al.,* 2008).

A strain *Bacillus subtilis* HM identified by 16S rDNA sequencing has been reported for aerobic decolourization of 8 different sulfonated azo dyes (Mabrouk and Yusef, 2008).

Seed germination and toxicity test for bioremediated effluent

Seed germination of was impacted by various concentrations (25% - 100%) of untreated textile effluent. At 25% of treated effluent germination was 22.2% after 24 hrs but at higher concentration of 50%, germination was 15.5% while 75% and 100% executed poisonous effect on seeds within 24 hrs (Figure 3D). After 72 hrs each conc. showed maximum activity. Lower conc. *i.e*. 25% and 50% showed excellent seed germination percentage while it abruptly decreased when conc. increased from 25% to 100%. Similar results were obtained by Ajmal and Khan (1985) that the germination of seeds

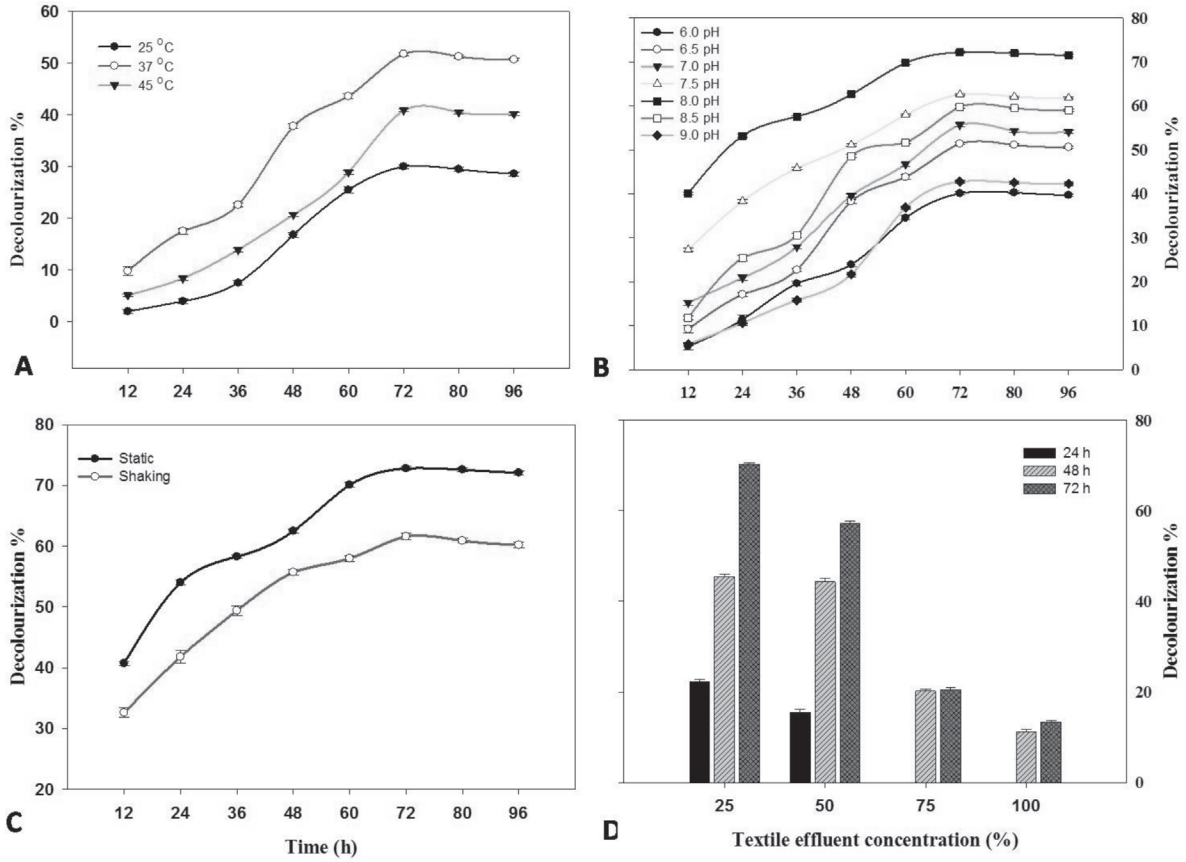

Fig. 3. Optimization of different decolourization process parameters: A. Effect of temperature; B. Effect of pH; C. Effect of aeration. D. Germination percentage at various concentration of textile effluent.

wasaffectedbadly when 75% and 100% concentrations of textile waste effluent, while no effect up to 50% concentration of textile effluent was noticed.

The toxicity test was performed at 100% concentration of effluent. Untreated effluent showed 30.3% seed germination and 3.03 germination speed (Table 1). On the other hand seed treated with treated effluent resulted % germination and germination speed of 98.7% and 10.8, respectively, for consortium of SPR_{42} and SPR_{48}. However, seed treated with *P. putida* bioremediated effluent showed 70.9% germination with a germination speed of 5.16. The emergency index, peak value and vigor index was (2.0, 3.03, 0.582); (9.8, 10.8, 595.7); (7.10, 5.16, 274.5) for control, consortium and *P. putida* respectively (Table 1). Seedling length was significantly stimulated by bioremediated effluent, while untreated effluent showed no significant effect of germination. Maximum increase in shoot length and root length was reported in seeds treated with consortium of isolates as compared to control and *P. putida* (Table 1). The shoot length was higher in case of consortium (6.21) as compared to control (0.067) and *P. putida* (3.110). On the other hand, root length was 0.160, 3.13 and 4.08 for control, *P. putida* and consortium, respectively (Table 1). Wet root weight (123.8), wet shoot weight (356), dry root weight (14.8) and dry shoot weight was (17.3) for seeds treated with consortium (Table 1). The effluent treated with consortium resulted best for seed germination as compare to untreated effluent.

Parshetti *et al.* (2006) observed similar results in phytotoxicity study, germination (%) of the seeds was fewer with Malachite Green dye treatment as relate to its degraded product and distilled water. Kaushik *et al*. (2005) studied the effect of untreated (0-100%) and treated textile effluent on seed germination (%), shoot length, root length, plant biomass and chlorophyll content of wheat. Chandra and Srivastava (2004) observed that raw effluent was more lethal to *P. aureus* than the treated effluent as concentrations > 5% had reduced the growth (shoot, root length and biomass). Mahmood *et al*. (2015) also found that bacterial consortium isolated from textile affected site was efficient in textile dye degradation and thus growth of *Zea maize* L. and *Sorghum Vulgare* Pers.

Conclusion

In our study we reported that decolourization of textile effluent by isolated aerobic bacterial consortium is because of biological activity of isolates. High decolourization degree and superficial conditions show the possible for this consortium to be used in the microbial treatment of dyeing mill effluents. Therefore, the further research will be required to know about the mechanism and end products of biodegradation of dyes. Thus, it can be suggested that microbial decolourization holds promise and can be utilised to progress a cost effective, eco-friendly microbial biotechnology technology for the bioremediation of textile dyes/effluent.

Conflict of interest

We hereby declare, there is no conflict of interest.

Data Availability

The data obtained during the present study is available with the corresponding author.

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