5.1 Introduction

Over the past decades, many microorganisms have been explored for degrading dyes including bacteria, filamentous fungi, yeasts, actinomycetes and algae (Syed et al., 2009). The features like omnipotence, faster growth, facultative nature and high adaptability are the desirable qualities of bacterial community for the bioremediation (Gomare et al., 2009). As per literature, the isolation work with azo dye degrading bacteria started in late 1970’s where species of *Bacillus* and *Aeromonas* were used. Numerous bacteria capable of dye decolourization, either in pure cultures or in consortia, have been reported (Junnarkar et al., 2006). In contrast to mixed cultures, the use of a pure culture provides several advantages like, predictable performance, detailed knowledge of the degradation pathways along with the enhanced assertion that catabolism of the dyes will lead to non-toxic end products under a given set of environmental conditions. Furthermore, bacterial strains and their activity can be monitored using culture-based or molecular techniques to compute population densities of the bacteria over time, which can then in turn be extrapolated to quantitatively analyse the kinetics of azo dye decolourization and mineralization (Khalid et al., 2010).

Different reports are available in the literature on the use of pure culture of *Bacillus* sp. for decolourization of azo dyes. Dave and Dave (2009) reported use of *B. thuringiensis* for decolourization of Acid Red 119. Similarly, Kolekar et al., (2008) reported the use of *B. fusiformis* for decolourization of Disperse Blue 79 and Acid Orange 10. Sharma et al., (2009) reported use of *B. subtilis* for decolourization of Acid Yellow 211. Aerobic decolourization of reactive azo dyes by *B. cereus* has been reported by Ola et al., (2010). Decolourization of different types of dyes has also been reported by genus *Bacillus* by various researchers (Wang et al., 2013; Anjaneya et al., 2011; Ayed et al., 2009; Dawkar et al., 2009; Panchamoorthy.
et al., 2009; Pourbabaee et al., 2006; Li et al., 2004). On the contrary, no reports are available in the literature on the use of pure culture of Bacillus pumilus for decolourization of azo dyes.

Bacterial degradation of dyes is usually initiated under static or anaerobic conditions by an enzyme catalysed reductive cleavage of azo bond leading to the formation of colourless aromatic amines. These toxic amines are in turn oxidized via deamination or hydroxylation reactions to simpler non-toxic forms under aerobic conditions only. Hence, attention is now focused on facultative anaerobic bacteria, which can perform high rate of dye decolourization under anoxic conditions along with the detoxification of the aromatic amines aerobically (Garg et al., 2012).
The present study focuses on the following objectives:

1. Optimization of process parameters for AB210 decolourization by *Bacillus pumilus* SRS83 at laboratory scale.
2. Characterization of AB210 dye biodegradation intermediates.
3. To propose a possible pathway for AB210 biodegradation by *Bacillus pumilus* SRS83.
4. Study on application of optimized parameters for colour removal of native textile dye effluent.
5. Toxicity studies to evaluate the toxicity of intermediates, by determining germination rates and biomasses of several plant species.
5.2 Materials and methods

52.1 Chemicals and media

The plant seeds viz. *Cicer arietinum* (Black gram) and *Vigna aconitifolius* (Turkish gram) were obtained from local markets. Microbial cultures of *E. coli*, *Azotobacter*, cellulose degrading and phosphate solubilising bacterial species were isolated from agricultural soil. The remaining chemicals and media used in this study are same as described in Chapter 4, section 4.2.1.

5.2.2 Dyes and effluent

Same as described in Chapter 4, section 3.2.1.

5.2.3 Phylogenetic analysis and sequence alignment

Same as described in Chapter 4, section 4.2.3.

5.2.4 Inoculum preparation

Same as described in Chapter 4, section 4.2.4

5.2.5 Physicochemical parameters

To evaluate the effects of operational and environmental conditions on the decolourization performance by the isolate A11, experiments were carried out at different pH values (6–12), initial dye concentrations (50–2000 mg/L), sodium chloride (NaCl) concentration (0.5-10% w/v), inoculum size (2x10^8– 10^9 cells/mL) as well as under static and shaking
speed (150 rpm). Further, the fed-batch process of decolourization by continuous additions of dye aliquots (50 mg/L) to culture media was also studied in the nutrient broth, under static condition without a supplement of additional nutrients.

If otherwise mentioned, all the experiments were performed in triplicates in 250 mL Erlenmeyer flask containing 100 mL nutrient broth (pH 7.0) as growth medium supplemented with 100 mg/L AB210 dye and 10% inoculum having $2 \times 10^8$ cells/mL. All the flasks were incubated at 32±2 °C under static condition. For liquid medium, the samples of the culture broth were analysed at the dye $\lambda_{\text{max}}$ 605 nm using the supernatant from the culture medium after centrifugation at 10,000 g for 10 min at 4 °C.

5.2.6. Estimation of reaction kinetics

5.2.6.1. Thermodynamic parameters

Arrhenius equation is the preferred method for incorporating the effect of temperature into complex mathematical models used to predict the fate of organic compounds released to the environment. Activation energy ($E_a$) can be determined by plotting the logarithm of the transformation rate versus the reciprocal of temperature, measured in Kelvin, and then determining the slope of the linear portion of the curve. Results can be safely extrapolated over the temperature range defined by the linearity of the Arrhenius plot.

The temperature dependence of the dye degradation rate constant was described by the Arrhenius equation (Eq. 5.1).

$$\lambda = Ae^{-\frac{E_a}{RT}}$$  \hspace{1cm} (5.1)
where, $\bar{\lambda}$ is the mean rate constant of the reaction at a particular temperature, $A$ is the pre exponential constant, $R$ is the gas constant and $T$ is the absolute temperature in Kelvin (Angelova et al., 2008).

5.2.6.2 Kinetic parameters

Batch experiments of AB210 dye decolourization were performed under optimum operating conditions with different initial dye concentrations and dye decolourization rate was determined. Assuming saturation kinetics with respect to biomass and carbon source, a Michaelis–Menten type expression with a substrate inhibition term, i.e., the Haldane model was used, as shown in Eq. 5.2.

$$V = \frac{V_{\text{max}} S}{K_m + S + \frac{S^2}{K_i}}$$

(5.2)

where, $V$ and $V_{\text{max}}$ are the initial volumetric dye decolourization rate (mg/L/h), $S$ is the initial dye concentration (mg/L), $K_m$ is the half velocity coefficient (mg/L) and $K_i$ is the inhibition coefficient (mg/L). The expression $V_{\text{max}}$ refers to the apparent $V_{\text{max}}$ value, i.e. the maximum initial volumetric decolourization rate (mg dye/L/h) under the experimental conditions (e.g., initial dye, temperature, pH and biomass concentration, etc.). The inhibition term ($K_i$) was included to account for a probable inhibitory effect of high dye concentrations on the dye decolourization rates. Consequently, a small $K_i$ value (comparable to that of the $K_m$ value) indicates inhibition of the dye decolourization due to the presence of high concentrations of dye, while a large $K_i$ value (much larger than the $K_m$ value) indicates that the presence of high concentrations of dye did not have a significant inhibitory effect on dye decolourization.
5.2.7 Preparation of cell free extract

Same as described in Chapter 4, section 4.2.6.

5.2.8 Enzyme assay

Same as described in Chapter 4, section 4.2.7.

5.2.9 Extraction and analysis of dye metabolites

5.2.9.1 UV-Vis spectrophotometry and FTIR analysis

Same as described in Chapter 4, section 4.2.8.1 and 4.2.8.2.

5.2.9.2 NMR and LC-MS analysis

To study the structural transformations in the dye molecule during the process of dye biodegradation, $^1$H-NMR spectra of the dye and its metabolites before and after treatment were recorded using a 300 MHz, NMR spectrometer (Bruker, Avance II 400 nm spectrometer, Switzerland).

LC-MS/MS analyses of dye intermediates and metabolites were carried out using the analytical C$_{18}$ column containing system coupled with an electrospray ionization (ESI) and an atmospheric pressure chemical ionization (APCI) (Waters, Micromass Q-Tof Micro™, USA). The analytes were eluted with a mobile phase of methanol-water ratio 70:30% at a flow rate of 0.15 mL/min. The column elute was introduced into the ESI source of the MS operated in the positive ion mode with full scan spectrum over the m/z scan range of 50–1400 with dry temperature 250 °C, capillary voltage 3250 V with a flow rate of 5.0 L/min for dry helium gas. Further, in
order to analyse the mass of compounds generated from LC-MS/MS, the degradation pathways and metabolites were hypothesized with reference to pathways illustrated in the MetaCyc database at http://metacyc.org/ and PathcaseKEGG database at and http://nashua.case.edu/PathwaysKegg/Web.

5.2.10 Toxicity studies

5.2.10.1 Microbial toxicity

Same as described in Chapter 4, section 4.2.9.1.

5.2.10.2 Phytotoxicity

Phytotoxicity studies were performed, in order to assess the impact of the treated coloured water on vegetation once it is discharged into the environment as well as to explore the probable reuse of the treated dye wastewater in irrigation fields. The tests were carried out for Cicer arietinum (chick pea), Vigna aconitifolius seeds, which are commonly used in Indian agriculture. Extracted AB210 metabolites were dried and dissolved in water to obtain the final concentration of 1500 mg/L and was used for phytotoxicity studies. The studies were carried out at room temperature by watering separately 10 mL samples of dye and its degradation products per day to seven seeds of each plant sowed in a watch glass. Control set was carried out using water at the same time. Germination (%) and the length of the plumule and radical were recorded after 8 days of incubation (Ayed et al., 2011; Shah et al., 2012).
5.2.11 Effects of C, N, P sources on effluent decolourization

Same as described in Chapter 4, section 4.2.10.

5.2.12 Textile dye effluent decolourization

Same as described in Chapter 4, section 4.2.11.

5.2.13 Statistical analysis

Same as described in Chapter 4, section 4.2.12.
5.3 Results and discussion

5.3.1 Decolourization of AB210 by *B. pumilus* SRS83

As can be seen from the photoplate 5.1 the untreated dye AB210 show dark greenish black colour whereas the flask of treated dye showed decolourization of the dye, the faint yellow colour which was due to the colour of the medium used in the study as the medium contains 1% peptone. The extent of the colour removed corresponds to 99% decolourization of the dye.

Photoplate 5.1 Flasks showing comparison of treated and untreated samples, with >99% decolourization of the control dye.

5.3.2 Phylogenetic position of isolates

Phylogenetic position of *B. pumilus* SRS83 in relation to the species of this genus is as illustrated (Fig. 5.1). The figure showed phylogenetic relationship of *B. pumilus* SRS82 and other related microorganisms found in
the GenBank database. The digits adjacent to nodes are the statistical frequency of the indicated species. The numbers shown in parentheses are accession numbers of different species. The optimal tree with the sum of branch length = 0.15110468 is shown. The 1150 bp sequence obtained is deposited in GenBank with accession no. JQ965500.1.

Fig. 5.1 Phylogeneic analysis of 16s rRNA gene sequence of B. pumilus SRS83. The percent numbers at the nodes indicate the levels of bootstrap support based on neighbor-joining analyses of 500 replicates. Brackets represents sequence accession numbers.
Chapter 5  Dye AB210 Biodegradation by B. pumilus SRS83

5.3.2 Effect of operational parameters

Microbial decolourization of model triazo recalcitrant dye AB210 by B. pumilus was studied in detail at different physico-chemical conditions (agitation, pH, inoculum size and repeated dye additions) and obtained results are shown in subsequent sections.

5.3.2.1 Effect of static and shaking condition

A complete decolourization of AB210 using the Bacillus SRS83 was obtained under static and microaerophilic condition after 18 h, but only 19% decolourization was achieved under shaking condition (Fig. 5.2). These results suggest that static condition was favourable for the decolourization, which could be due to involvement of azoreductase for the decolourization of azo dyes. Hence, in this study static conditions were adopted to investigate the bacterial decolourization. Reportedly, many bacteria reduce the highly electrophilic azo bond (–N=N–) in the dye molecule by low specificity cytoplasmic azoreductase resulting in production of colourless aromatic amines, under anoxic/static conditions and is normally inhibited by the presence of oxygen primarily due to competition in the oxidation of reduced electron carriers like NADH with either oxygen or azo groups as an electron acceptor (Garg et al., 2012).

5.3.2.2 Effect of pH

It is important to study the effect of pH on decolourization process, as transport of dye molecule into the cell and its solubilisation is pH dependent and thought to be rate limiting step for decolourization of dyes (Agrawal et al., 2013). The culture was able to decolourize the dye in a broad range of pH however, optimum pH for dye decolourization was
Fig. 5.2 Effect of incubation under static and agitation (150 rpm) on decolourization of AB210 by *B. pumilus* SRS83 with function of time.

Fig. 5.3 Effect of different pH on decolourization of AB210 by *B. pumilus* SRS83 with function of time.
was found to be 8.0 (Fig. 5.3). Decrease in percentage decolourization was also observed at lower pH (6-7) as well as higher pH (9-12) for the cultures. The time required for complete decolourization was increased with increasing order of pH. Here the results demonstrated that alkaline pH was not changed even after complete decolourization of the dye, while acidic pH 6 was brought to neutral during the decolourization process. This indicates that the isolated strain can be used effectively to treat the textile effluent, which generally varies in pH levels. Garg et al., (2012) and Tripathi and Srivastava (2011) also made similar observations.

### 5.3.2.3 Effect of salt concentration

The high salt concentration, up to 15–20%, is a consequent product of batch processes in both the dye-consuming industries (Anjaneya et al., 2011). Thus, it was necessary to study the effect of salt concentration on azo dye decolourization. Exposure of strain to increasing salt concentration showed significant growth and 87.04% decolourization, up to 5% of salt concentration. Increasing salt concentration showed slight decrease in decolourization efficiency, with 73.54% decolourization at 7.5% salt concentration (Fig. 5.4). Decolourization efficiency was found to be reduced as the salt concentration increased gradually up to 10%. Generally, sodium concentration above 3 g/L inhibits bacterial activities because of plasmolysis or loss of activity of cells. The ability of the strain to tolerate salt concentration up to 5% without significant decrease in decolourization efficiency makes it competent for dye decolourization. The change in dye decolourization rates with increasing salt concentrations may be attributed to the variation of enzyme activity or changes in the molecular transportation through the cellular membrane of microorganisms by higher salt conditions. Anjaneya et al., (2011) and Meng et al., (2012) also reported similar observations.
5.3.2.4 Effect of initial bacterial biomass

As shown in (Fig. 5.5), AB210 reduction required the presence of bacterial cells, and faster decolourization was observed in the presence of higher initial cell concentration. About 12.51% and 99.45% of AB210 were removed within 60 min when the inoculum size was increased from $10^8$ to $10^9$ cells/mL, respectively. Over 18.73, 43.12, 61.87 and 76.43% decolourization was achieved for systems containing 2, 4, 6 and $8\times10^8$ cells/mL, respectively. Meng et al., (2012), also noticed an increase in the decolourization rate with an increase in inoculum size for decolourization of 0.2 mM AR27 dye by *Shewanella aquamarine*. Shah et al., (2012) found an increase in decolourization rate up to 6% inoculum ($6\times10^6$ cells/mL) for decolourization of 400 mg/L of RO13 dye by *Alcaligenes faecalis* PMS-1 and 200 mg/L of Orange 3R dye by *Klebsiella* sp. respectively.
5.3.2.5 Fed-batch decolourization

Repeated use of developed cell biomass supports economical utilization of resources in decolourization process and subsequently for industrial purpose. In the present study the decolourization efficacy of the B. pumilus by the repeated dye addition have been analysed. The organism was able to retain its efficiency up to 6 repeated dye addition cycles with little variation in percentage decolourization as well as time requirement (Fig. 5.6). The decreasing percentage decolourization was observed from 7th to 12th cycle with increase in the time required for decolourization. Furthermore, addition of dye in the 13th cycle dose doesn't show any decolourization. The observed gradual decrease in decolourization might be due to the culture entering into the stationary phase and subsequently into the death phase, resulting in the inhibition of enzyme systems gradually or probably due to the scarcity of the available nutrient in the medium or due to accumulation of toxic dye metabolites in the medium.

Similar experimental results were reported by Dave and Dave (2009) where B. thuringiensis took 6 h to decolourise first dose of 300 mg/L of Acid Red 119 but then up to 900 mg/L addition, an increase in decolourization activity was noticed with decrease in time taken for due to increased population of decolourizing bacterial culture. Thereafter, gradually the time for decolourization increased, probably due to either attainment of stationary or decline phase of the growth by the culture or due to the scarcity of the available nutrients in the medium. The highest decolourization rate 218.0±2.0 mg/L/h was observed for a second and third dose of addition. Similarly, Telke et. al., (2009) noticed decolourization of repeated addition of Reactive Red 141 dye by Rhizobium radiobacter up to six cycles with variable decolourization rate (74–90%).
Fig. 5.5 Effect of different inoculum size on decolourization of AB210 by B. pumilus SRS83.

Fig. 5.6 Fed batch decolourization of AB210 by B. pumilus SRS83.
5.3.3 Estimation of activation energy ($E_a$)

The microorganisms are ectothermic and hence act in response to temperature change by adaptation to changed temperature value through biochemical mechanisms, which being enzymatic are influenced by the temperature change. Therefore, temperature is a factor of primary significance for all processes associated with microbial vitality including the remediation of water and soil (Angelova et al., 2008).

Extent of decolourization depends on the activation energy ($E_a$) of the reaction, which was estimated by Arrhenius equation. The plot of $-\ln \left( \frac{C_t}{C_0} \right)$ against time (Fig. 5.7), according to Eq. 5.1, provides a useful tool for visual control on the stability of the decolourization process. If the decolourization follows the first order exponential decay pattern then the data forms a straight line with the value of slope equals to the value of the decolourization rate ($\lambda$). It is evident as well that, $B. \ pumilus$ SRS83 is not as tolerant to high temperatures; at 60 and 70 °C the decolourization of AB210 with this strain although starts with a rate higher than expected one but stops completely after 6 h incubation most probably because of a thermal inactivation of the cells. At these higher temperatures and under the reaction conditions, which have limited availability any source of carbon, can probably provoke partial autolysis of the cells, which along with the very low concentration of AB210 is sufficient to interfere with the colorimetric estimation of dye decolourization. Therefore, only the experimental points obtained within the time ranges indicated with solid lines, were selected for further consideration. The plot of $-\ln \lambda$ against $\frac{1}{T}$ results in a straight-like graph, as shown in Fig. 5.8. The high-degree of linearity ($R^2 = 0.98$) gives reliable estimations of the activation energy ($E_a$) and frequency factor ($A_o$). The obtained values were $E_a = 37.91$ J/mol (9.05 cal/mol) and $A_o = 3.65 \times 10^5$ h$^{-1}$. As reported that the general activation energy range of enzyme-catalysed reactions are usually within 4-20 cal mol$^{-1}$ (Shah et al., 2012).
Fig. 5.7 Decolourization rates of AB210 *B. pumilus* SRS83 after 24 h at different temperatures with function of time.

Fig. 5.8 Estimation of activation energy for AB210 decolourization rate by *B. pumilus* SRS83 using Arrhenius plot.
5.3.4 Kinetic modelling of AB210 decolourization

Considering *Bacillus pumilus* cells as a whole cell enzyme, kinetic modelling of azo dye decolourization was performed using the data set obtained from the assay testing the effect of different initial dye concentration (50 to 2000 mg/L) on dye decolourization kinetics. Although, at a higher dye concentration (> 500 mg/L) the decolourization rate observed was less, probably due to the toxic effect of dyes and/or inadequate biomass concentration for the uptake of higher concentrations of dye, as well as blockage of active sites of azoreductase by dye molecule with different structure.

Initial volumetric decolourization rates for AB210 were determined using linear regression at each different initial AB210 concentration series. Subsequently, the initial volumetric decolourization rates were correlated statistically using a least-square, non-linear regression with the corresponding initial dye concentration according to Eq. 5.2. From the non-linear regression (Fig. 5.9), the values for $V_{\text{max}}$, $K_m$ and $K_i$ values 11.36±1.5 mg/L/h, 63.92±2.93 mg/L, and 977.77±5.7 mg/L ($R^2 = 0.99$), respectively. The model predicted initial AB210 decolourization rate, without any inhibition (i.e. $K_i = \infty$) at an initial AB210 concentration of 1000 mg/L, is 5.67 mg/L/h as opposed to the experimentally observed (i.e., inhibited) AB210 decolourization rate of 5.28 mg/L/h. A relatively high initial AB210 concentration of 1500 mg/L resulted in a moderate degree of inhibition of the dye decolourization process as is also shown by the relatively high $K_i$ value as compared to the $K_m$ value. The experimental data obtained for AB210 decolourization at an initial concentration of 200 mg/L were used to illustrate the Monod’s model fit. From the non-linear regression, the values for $V_{\text{max}}$ and $K_m$ were determined as 10.63±1 mg/L per day, and 19.01±0.89 mg/L, respectively ($R^2 = 0.97$).
As per literature, a similar analysis was performed with initial volumetric decolourization rate data obtained with the mixed, methanogenic culture on dye Reactive Red 2 at a concentration range of 50–2000 mg/L. The values for $V_{\text{max}}$, $K_m$, and $K_i$ obtained were as 109±5 mg/L/d, 8.6±3.9 mg/L, and 7285±25.17 mg/L ($R^2 = 0.99$), respectively. As reported, the initial volumetric decolourization rate was actually higher for the dyes with higher molecular weight supporting a widely accepted mechanism, in which azo dye decolourization takes place extracellularly, via electron transfer mediation (Beydilli and Pavlostathis, 2005).

![Degradation kinetics for AB210 by B. pumilus SRS83 under inhibited and uninhibited and experimental conditions.](image)

**Fig. 5.9** Degradation kinetics for AB210 by *B. pumilus* SRS83 under inhibited and uninhibited and experimental conditions.

### 5.3.5. Enzyme analysis

Induction of various enzymes during decolourization gives additional insight of decolourization mechanism and also supports the active role of
microorganisms in the biodegradation process. Enzyme activities during
decolourization of AB210 by *Bacillus* SRS83 are summarized in Table 5.1.
Significant induction in the intracellular lignin peroxidase (512%),
azo reductase (292%) and intracellular and extracellular tyrosinase activity
(280% and 500%, respectively) was noticed as compared to control.
Likewise, there was a moderate induction in intracellular laccase (146%)
and reductive NADH-DCIP reductase (276%).

These results explain the synergic action of these enzymes in dye
biodegradation. Moreover, bacterial degradation of dyes under static
conditions often involves reductive cleavage of azo bond as the initial step.
In this study, significant induction of azo reductase and NADH-DCIP
reductase activities suggests their involvement in decolourization of dye
molecule.

Table 5.1
Extracellular and intracellular enzyme activity during decolourization of
AB210 by *B. pumilus* SRS83.

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Control</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Enzyme activity (µM/min/mg protein)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>Test</td>
</tr>
<tr>
<td>Laccase</td>
<td>3.84 ± 0.12</td>
<td>5.63 ± 0.08</td>
</tr>
<tr>
<td>Tyrosinase</td>
<td>71.48 ± 2.21</td>
<td>200.67 ± 19.23</td>
</tr>
<tr>
<td>Intracellular Tyrosinase</td>
<td>3.01 ± 0.65</td>
<td>15.05 ± 1.37</td>
</tr>
<tr>
<td>Extracellular Tyrosinase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lignin peroxidase</td>
<td>2.30 ± 0.42</td>
<td>11.78 ± 0.29</td>
</tr>
<tr>
<td>Intracellular Lignin peroxidase</td>
<td>3.77 ± 0.42</td>
<td>10.98 ± 0.23</td>
</tr>
<tr>
<td>Extracellular Lignin peroxidase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NADH-DCIP reductase</td>
<td>1.96 ± 0.23</td>
<td>5.41 ± 0.77</td>
</tr>
<tr>
<td>Azoreductase</td>
<td>1.38 ± 0.18</td>
<td>4.03 ± 0.41</td>
</tr>
</tbody>
</table>

Control= enzyme extracted from culture medium without dye after 18 h; Test = enzyme
extracted from the dye decolourized culture medium after 18 h. Values are mean of three
experiments ± SD. Percent induction calculated from mean values.
3.6. Evaluation of decolourization and biodegradation of AB210

3.6.1. UV–Visible spectral analysis

Evidence for the removal of the dye can be observed with absorbance at dye $\lambda_{\text{max}} = 605$ nm, being virtually zero after 18 h and an increase in absorbance towards the UV region (Fig. 5.10). After shaking treatment, reduction in absorbance in UV region is noticed. These results indicate that the colour removal by *Bacillus pumilus* SRS83 may be largely attributed to the biodegradation.

![UV-VIS spectra of AB210 dye before and after treatment with *B. pumilus* SRS83 under static and shaking condition.](image)

**Fig. 5.10** UV-VIS spectra of AB210 dye before and after treatment with *B. pumilus* SRS83 under static and shaking condition.
5.3.6.2. FTIR spectrum

Comparison of FTIR spectrum of control dye (Fig. 5.11a) with degraded metabolites after 18 h of treatment (Fig. 5.11 b), clearly indicated the biodegradation of dye by B. pumilus. Peaks in control spectrum showed O-H stretch at 3516.35 cm\(^{-1}\), the peak at 1597.11 cm\(^{-1}\) showed N=N stretch (indicates the azo bonds present in dye), The presence of N=O\(_2\) stretch at 1489.10 cm\(^{-1}\) and 1556.61 cm\(^{-1}\) confirmed the aromatic nitro compound, N-H deformation of primary and secondary amines was observed at the peak of 1649.19 cm\(^{-1}\), O-H deformation was observed at 1411.9 cm\(^{-1}\), S=O stretch was observed at the peak of 1330.93 cm\(^{-1}\), 1174.69 cm\(^{-1}\), 1139.97 cm\(^{-1}\) (supports the dye contains the sulfonated dye compound), peak 1294.63 cm\(^{-1}\) shows C-N vibration, 1045.45 cm\(^{-1}\) indicates C-OH stretching of primary alcohol and peak at 842.92 cm\(^{-1}\) indicates C-H deformation of a benzene ring. C-H stretching is also indicated by peaks 2924.18 cm\(^{-1}\) and 2854.74 cm\(^{-1}\).

The FTIR spectra of extracted metabolites after 18 h of treatment showed disappearance of sharp peak at 1597.11 cm\(^{-1}\) which is specific for azo compounds, which confirms cleavage of azo bonds of the dye. The peak at 3225.09 cm\(^{-1}\) with supporting peak at 3373.61 cm\(^{-1}\) and near 1654.98 cm\(^{-1}\) indicates N-H stretching of amines. C=C stretching of aromatic compound forms peak at 1458.23 cm\(^{-1}\), 1307.78 cm\(^{-1}\) and 1149.61 cm\(^{-1}\) shows S=O stretching of sulphonamides and sulfonic acids respectively. Peak at 831.35 cm\(^{-1}\) shows C-H deformation of trisubstituted benzene, while those at 3066.92 cm\(^{-1}\) corresponds to the C-H stretching of homocyclic aromatic compounds, 2603.99 cm\(^{-1}\) corresponds to S-H stretching of organo sulphur compounds, 1676.20 cm\(^{-1}\) C=O stretching of O-hydroxy benzoates, 1556.61 and 1541.18 indicates NO\(_2\) stretching of saturated, primary and secondary nitro compounds, respectively. The appearance of new peaks in
degraded products and the disappearance of peaks from the control spectrum confirm the degradation of dye.

Sequential shaking treatment after static decolourization further enhanced the mineralization of dye as is supported by the FTIR data obtained. The peak at 3423.76 corresponds to O-H stretching of aromatic alcohols and the peaks at 2926.11 cm\(^{-1}\), 2958.90, and 1454.38 cm\(^{-1}\) corresponds to C-H stretching of alkanes. The peaks at 1303.78 cm\(^{-1}\) and 1656.91 cm\(^{-1}\) indicates C=C stretching of alkenes. The lack of peak at 1597 cm\(^{-1}\), which was present in the control AB210 indicates the breaking of azo bond by azoreductase.

5.3.6.3. NMR spectroscopy data

\(^1\)H-NMR spectrum of the AB210 dye shows broad multiplets corresponding to aromatic components at δ 8.31–8.14, doublets at δ 6.0, 7.4 and 7.5 and singlet at δ 5.22, 5.9, 7.8 and 7.9 (Fig. 5.12 a). The aromatic proton at δ 7.4–7.8 corresponds to –NH and two doublets at 6.0δ and 5.9δ accounts for the presence of two adjacent protons on the aromatic ring.

In order to understand clearly whether the AB210 has undergone effective degradation or not, \(^1\)H NMR spectrum of the product was also recorded using the same NMR spectrometer. The product exhibited three singlet at 7.71δ, 6.97δ and a doublet at 6.73δ, which reveal the presence of the proton of –SO\(_3\)H, aromatic and imino proton, respectively. In addition to these signals, the product also gave peaks appearing in the \(^1\)H NMR spectra of the ethyl acetate-extracted metabolites had signals (δ=6.73– 7.71) different from the parent dye, multiplets at δ 7.71–7.57, 4.22-4.08, 3.61-3.45, 2.00-1.89, 1.69-1.39, 1.02-0.85 and doublets were observed at δ 6.71, 6.9, 7.2 and singlet at δ 8.0-8.26 (Fig. 5.12 b and c).
Fig. 5.11 FTIR spectra of (a) AB210 dye and (b) FTIR spectra of dye after static dye decolourization and further transformation under shaking condition by B. pumilus SRS83.
While no aromatic signals were observed in extract obtained after shaking, signals ($\delta=3.0-5.0$) different from the parent dye (Fig. 5.12 d and e) in comparison with the $^1$H-NMR spectrum of the AB210 dye, indicating to complete biotransformation of the dye molecules into metabolic intermediates with signals completely different from the AB210 dye, as described above. Sharma et al., (2004) and Sarayu and Sandhya, (2010) reported similar observations upon microaerophilic treatment of dye for Acid Violet 17 by a bacterial consortium and aerobic treatment Remazol Orange by *Pseudomonas aeruginosa* respectively.

![Fig. 5.12 NMR spectra of (a) AB210 dye.](image-url)
Fig. 5.12 NMR spectra of AB210 dye after (b, c) static dye decolourization and (d,e) further transformation under shaking condition upon treatment by B. pumilus SRS83
5.3.7. LCMS analysis and proposed pathway

LC-MS/MS analysis was conducted to investigate the dye intermediates formed after decolourization of dye AB210 and further biotransformation under shaking conditions by *B. pumilus* SRS83. The compounds and their respective mass were interpreted from both positive and negative ionization mass spectra (Table 5.2). Decolourization of AB210 was carried out under static condition suggesting reductive degradation as a preliminary step in AB210 biodegradation (Fig. 5.13a and b). Dye AB210 initially undergoes symmetric reductive cleavage to produce primary aromatic and sulfonated aromatic amines, namely benzene1, 2, 4-triamine, 4-nitroaniline, 4-amino-N-(4-aminophenyl) benzenesulfonamide and 3, 4, 6-triamino-5-hydroxynaphthalene-2, 7-disulfonic acid, which is also supported by significant induction in azoreductase activity as shown in Table 5.1. Microbial degradation of azo dyes via the reduction of azo bonds catalysed by azoreductases has been extensively reported (Chan et al., 2012; Kadam et al., 2011). Further reductive biotransformation of 4-amino-N-(4-aminophenyl) benzenesulfonamide and 3, 4, 6-triamino-5-hydroxynaphthalene-2, 7-disulfonic acid was observed under microaerophilic condition and this could involve reductive deamination and desulfonation pathways. Chan et al., (2012) proposed deamination and desulphonation as the degradation mechanisms during dye biotransformation while analysing the action of *Alcaligenes faecalis* PMS-1 for Reactive Orange 13 dye degradation. Reductive deamination has been proposed as a novel preliminary step in anaerobic biodegradation of halogenated anilines by (Travkin et al., 2002; Chan et al., 2012) while working with *Rhodococcus* sp. *In silico* analysis of genes from *B. pumilus* revealed the presence of the gene which codes for sulphuric ester hydrolase enzyme with a possible sulfatase activity. Therefore, these along with laccase, could contribute to the deamination and desulphonation process.
Table 5.2
Metabolic intermediates detected by LC-MS/MS analyses after decolourization of AB210 by *B. pumilus* SRS83

<table>
<thead>
<tr>
<th>M. W.</th>
<th>m/z (amu)</th>
<th>Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>123.15</td>
<td>120.1</td>
<td>Benzene 1,2,4 triamine</td>
</tr>
<tr>
<td>263.31</td>
<td>261.2</td>
<td>4-amino-N-(4-aminophenyl)benzenesulfonamide</td>
</tr>
<tr>
<td>334.32</td>
<td>333.2</td>
<td>3,4,6-triamino-5-hydroxynaphthalene-2,7-disulfonic acid</td>
</tr>
<tr>
<td>138.12</td>
<td>138.1</td>
<td>4-Nitroaniline</td>
</tr>
<tr>
<td>126.11</td>
<td>ND</td>
<td>Benzene 1,2,4 triol</td>
</tr>
<tr>
<td>265.28</td>
<td>267.1</td>
<td>4-hydroxy-N-(4-hydroxyphenyl)benzenesulfonamide</td>
</tr>
<tr>
<td>272.23</td>
<td>271.1</td>
<td>3,5,6,7-tetrahydroxynaphthalene-2-sulfonic acid</td>
</tr>
<tr>
<td>139.10</td>
<td>138.1</td>
<td>4-Nitrophenol</td>
</tr>
<tr>
<td>110.11</td>
<td>109.1</td>
<td>Benzene^1,4-diol</td>
</tr>
</tbody>
</table>

After further biotransformation of AB210 by *B. pumilus* under shaking condition for 30 h at 37±2 ºC.

<table>
<thead>
<tr>
<th>M. W.</th>
<th>m/z (amu)</th>
<th>Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>122.12</td>
<td>120.1</td>
<td>Benzoate</td>
</tr>
<tr>
<td>110.11</td>
<td>109.1</td>
<td>Catechol</td>
</tr>
<tr>
<td>142.11</td>
<td>141.1</td>
<td>Cic-cis Muconate</td>
</tr>
<tr>
<td>160.12</td>
<td>159.0</td>
<td>3-oxoadipate</td>
</tr>
<tr>
<td>304.23</td>
<td>306.2</td>
<td>2-[(E)-2-carboxy-2-hydroxyethenyl]-4-hydroxy-5-sulfo benzoic acid</td>
</tr>
<tr>
<td>224.16</td>
<td>ND</td>
<td>2-[(Z)-2-carboxy-2-hydroxyethenyl]-4-hydroxybenzoic acid</td>
</tr>
<tr>
<td>154.12</td>
<td>153.1</td>
<td>Protocatechu ate</td>
</tr>
<tr>
<td>188.1</td>
<td>186.1</td>
<td>3-carboxy-cis-cis-muconate</td>
</tr>
<tr>
<td>186.1</td>
<td>185.1</td>
<td>2-carboxy-5-oxo-2,5-dihydro-furan-2-acetate</td>
</tr>
<tr>
<td>142.11</td>
<td>144.1</td>
<td>2-oxoadipate-enol-lactone</td>
</tr>
<tr>
<td>160.12</td>
<td>159.0</td>
<td>3-oxoadipate</td>
</tr>
<tr>
<td>162.18</td>
<td>159.1</td>
<td>Cinnamic acid</td>
</tr>
<tr>
<td>114.09</td>
<td>ND</td>
<td>2-oxopent-4-eneoic acid</td>
</tr>
</tbody>
</table>
Chapter 5  Dye AB210 Biodegradation by B. pumilus SRS83

of sulfonated dye intermediates. An FMN-dependent NADH-azoreductase has been reported by Gioia et al., (2007) during in silico analysis of B. pumilus SAFR 03. Additionally, a CotA-type laccase from Bacillus pumilus is reported to have good ability to decolourize dye indigocarmine (Reiss et al., 2011). Significant lignin peroxidase activity from B. pumilus has been reported earlier by Sethi et al., (2013).

The metabolites formed during the shaking biodegradation process AB210 were also analysed and the biodegradation pathways are described. The degradation of the three dye metabolites viz. benzene 1, 2, 4 triamine, 4-amino-N-(4-aminophenyl)-benzene sulfonamide and 4-nitroaniline probably involves shaking degradation through benzoate degradation pathway. From the Kegg pathway database, B. pumilus was known to possess benzoate degradation pathways, which is further confirmed by LCMS data. Additionally, the biotransformation of benzene 1,2,4 triamine to benzene 1,2, 4 triol is further supported by various reports of existence of deaminases in B. pumilus sp. as also reported for Bradyrhizobium sp. strain JS329 by Qu and Spain, (2011). The degradation mechanism of 3,5,6,7-tetrahydroxynaphthalene-2-sulfonic acid probably involved oxygenases, which function in aromatic ring cleavage, producing intermediates including 2-[(E)-2-carboxy-2-hydroxyethenyl]-4-hydroxy-5-sulfobenzoic acid, 2-[(Z)-2-carboxy-2-hydroxyethenyl]-4-hydroxy-5-sulfobenzoic acid, and 2-[(Z)-2-carboxy-2-hydroxyethenyl]-4-hydroxybenzoic acid. It is possible to hypothesize that the 2-[(Z)-2-carboxy-2-hydroxyethenyl]-4-hydroxybenzoic acid formed undergone successive biotransformation and mineralization via protocatechuate degradation pathways. LCMS data support the predominance of meta-cleavage protocatechuate pathway during mineralization of dye AB210 resulting in detection of metabolites including 2-oxo-2H-pyran-4,6-dicarboxylic acid, 4-carboxy-4-hydroxy-2-oxoadipate and pyruvate (Table 5.2), which eventually enter the TCA cycle.
Fig. 5.13 (a) Proposed degradation pathway of AB210 dye by *B. pumilus* SRS83.
On the contrary, most amines formed during the anaerobic reduction of the azo dyes are unstable under shaking conditions as the compounds could undergo auto-oxidation to produce recalcitrant intermediates as proposed by Stolz (2001) and Chan et al., (2012). Under static condition 3,5,6,7-tetrahydroxynaphthalen- e-2-sulfonic acid contains a hydroxy group in the ortho- position to the amino group, which could undergo auto-oxidation in the presence of oxygen to form 7-hydroxy-3,4 dioxo-3,4 dihydronaphthalene-2,7-disulfonic acid which then subsequently reacts to form cinnamic acid and 2-oxopentenoate as traced in samples from AB210 decolourization (Table 5.2). The biotransformation pathway for 3, 5, 6, 7-tetrahydroxynaphthalene-2-sulfonic acid is illustrated in Fig. 5.14. These observations demonstrated that there is a high possibility for mineralization to occur under shaking degradation of dye products, by *B. pumilus* sp. SRS83.

![Fig. 5.13 (b). Proposed benzoate degradation pathway of AB210 dye by *Bacillus pumilus* SRS83](image)
Fig. 5.14. Proposed autooxidation and oxidative degradation pathway for dye metabolite (3,4,6-triamino-5-hydroxynaphthalene-2,7-disulfonic acid).
5.3.8. Toxicity study

5.3.8.1. Phytotoxicity

The relative sensitivity of the two plant seeds against the untreated dye and dye metabolites extracted after complete decolourization are listed in Table 5.3. The untreated dye at 1500 mg/L concentration showed 30% and 60% germination inhibition in *Cicer arietinum* and *Vigna aconitifoilus* seeds, respectively. There was no germination inhibition in both the plants by AB210 metabolites at 1500 mg/L concentration. The shoot and root growths were as good as their growth in control set. Similar results are reported by Shah et al., (2012) while studying the detoxification effect, on *O. sativa, V. radiate, S. bicolor* and *T. aestivum* plants, of the metabolites of dye Reactive Orange 13 upon biodegradation by *Alcaligenes faecalis* PMS-1.

5.3.8.2. Microbial toxicity

Microbial toxicity studies on *E. coli, Azotobacter, cellulose degrader* and phosphate solubilising bacteria showed variable zones of inhibition (Table 5.3) surrounding the well containing the dye, while the degraded products did not showed an inhibitory zone, which confirmed the non-toxic nature of the extracted metabolite.

These results are consistent with that obtained by Gomare et al., (2009) upon biodegradation of Golden Yellow HER by *Brevibacillus laterosporus* MTCC 2298, and Ayed et al., (2011) upon biodegradation of Methyl Red by an isolated *Sphingomonas paucimobilis*. Meng et al., (2012) also reported detoxification upon biodegradation of Acid Red 27 by *Shewanella aquimarina* for microbial and phytotoxicity studies.
### Table 5.3
Toxicity study of AB210 dye and its metabolites after treatment with *B. pumilus* SRS83.

<table>
<thead>
<tr>
<th>Parameter analyzed</th>
<th>Test organism</th>
<th>Control</th>
<th>AB210&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Metabolites &lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microbial toxicity (Zone of inhibition, cm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zone of inhibition</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>0.80 ± 0.01</td>
<td>1.00 ± 0.02</td>
<td>0.81 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>Azotobacter sp.</td>
<td>0.80 ± 0.01</td>
<td>1.1 ± 0.057</td>
<td>0.88 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>Phosphate-solubilizer</td>
<td>0.80 ± 0.02</td>
<td>0.93 ± 0.015</td>
<td>0.81 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>Cellulose degrader</td>
<td>0.81 ± 0.03</td>
<td>1.00 ± 0.04</td>
<td>0.83 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>Phytotoxicity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cicer arietinum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Germination %</td>
<td>100</td>
<td>70</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Root (cm)</td>
<td>3.10 ± 0.42</td>
<td>0.89 ± 0.75</td>
<td>3.01 ± 0.30</td>
<td></td>
</tr>
<tr>
<td>Shoot (cm)</td>
<td>3.06 ± 0.37</td>
<td>0.83 ± 0.60</td>
<td>2.91 ± 0.32</td>
<td></td>
</tr>
<tr>
<td>Vigna aconitifolius</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Germination (%)</td>
<td>100</td>
<td>60</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Root (cm)</td>
<td>4.14 ± 0.41</td>
<td>0.79 ± 0.58</td>
<td>3.92 ± 0.39</td>
<td></td>
</tr>
<tr>
<td>Shoot (cm)</td>
<td>2.19 ± 0.28</td>
<td>0.66 ± 0.61</td>
<td>2.16 ± 0.31</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> 1500 mg/L; values are mean of three experiments and SD (±) is significantly different from the control at, *P<0.001, by one-way analysis of variance (ANOVA) with Tukey-Kramer comparison test.
5.3.9 Effluent decolourization

In order to prove the utility of the culture for the treatment of industrial waste containing various acid azo dyes and for degradation of mixtures of such dyes in the effluent studies on biotreatment of textile industry effluent by *Providencia* sp. SRS82 were conducted. As the effluents from textile industry have high COD therefore to enhance the decolourization and degradation of the effluent studies on optimization of nutrient supplements were also conducted.

5.3.9.1. Effects of varying carbon sources on decolourization of effluent

In order to optimize the nutrient composition for enhanced decolourization, COD and BOD reduction of textile effluent by using *B. pumilus* SRS83 and *Providencia* sp. SRS82 and their consortium-BP; effect of additional carbon, inorganic nitrogen and phosphorus sources was studied at static conditions as no considerable decolourization performance was observed under nutrient limited conditions.

Biodegradation activity of the isolate varied greatly according to the type of carbon and nitrogen sources used in the Bushnell Haas medium. As depicted in Fig. 5.15, in the Bushnell Haas Medium (control), only 8.3%, decolourization of the effluent was observed over 24 h with *B. pumilus* sp. SRS83. In an attempt to enhance decolourization in the control medium, the medium was supplemented with extra carbon and nitrogen and phosphorus sources. Maximum (72.11%) dye decolourization by *B. pumilus* sp. SRS83 within 24 h was observed with cometary starch in comparison to that with
glucose (69.42%), lactose (62.18%) and sucrose (66.56%).

The low decolourization extent at lower than optimum C-source concentration could be attributed to insufficient level of utilizable C-source for meeting the growth requirement for colour removal by the two isolates. Starch is an easily metabolizable carbon source for *Bacillus* sp. due to presence of enzyme amylase and it not only acts as a reducing agent for dyes, but also promotes bacterial growth and metabolism, thereby increasing dye decolourization.

5.3.9.2. Effects of varying N and P sources on effluent decolourization

Reports have shown that decolourization of synthetic textile dye effluent was best in carbon as well as nitrogen-sufficient medium along with a twofold increase in biomass (Mathew and Madamwar, 2004).

Among the inorganic nitrogen sources, sodium nitrate effected dye decolourization of 87.21% within 24 h by *B. pumilus* sp. SRS83 (Fig. 5.16). Other nitrogen sources may be arranged in the following decreasing order of dye decolourization: ammonium nitrate (81.43%), ammonium sulphate (82.12%) > urea (76.13%) > potassium nitrate (78.43%).

Similarly, combination of sodium dihydrogen phosphate and disodium hydrogen phosphate showed enhanced dye decolourization as compared to potassium dihydrogen phosphate dipotassium hydrogen phosphate showed 93.75% decolourization of effluent by *B. pumilus* sp. SRS83 and therefore, were selected as the best source of phosphate for effluent decolourization (Fig. 5.17).
Fig. 5.15 Effect of different carbon sources on effluent decolourization. (1-BHM/Control, 2-Glucose, 3-Sucrose, 4-Lactose, 5-Starch).

Fig. 5.16 Effect of addition of different nitrogen sources on effluent decolourization. (1-BHM, 2-NaNO₃, 3-NH₄NO₃, 4-Urea, 5-(NH₄)₂SO₄, 6-KNO₃).
5.3.9.3. Optimization of carbon, nitrogen and phosphorus content

Often, biodegradation of pollutants is found to be limited by the availability of nitrogen. The organism may have insufficient nitrogen source at lower concentration of nitrogen source or may influence the change in pH at higher nitrogen content. Thus, both low and high nitrogen source concentrations have an adverse effect on dye decolourization.

The maximum decolourization of 90.93% for the effluent was noticed at 10 g/L concentration of glucose concentration for Bacillus sp. SRS82 as shown in Fig. 5.18. The percentage decolourization of dye for different nitrogen source concentrations is shown in the Fig. 5.19. Similarly, the maximum decolourization of 94.33% for the effluent was noticed at 4 g/L concentration of sodium nitrate. In order that during effluent decolourization by B. pumilis SRS83, the system not being deficient in available organic material and nitrogen, carbon, nitrogen and phosphorus sources were added. As shown in Fig. 5.20, it was observed that maximum decolourization of dye occurred at a phosphorus content of 0.2 g/L (95.8%), below which the organisms have insufficient amount of phosphorous for its growth, and further high concentration of phosphorous does not support the growth. Thus, the involvement of small amount of phosphorus enhanced dye biodegradation as proved by Velan et al., (2012).

5.3.9.4 Effluent Treatment

The textile industry effluent usually have a low BOD/COD ratio (0.26) and therefore the contents that could not be easily degraded. Hence stabilization of the textile industry effluent was carried out using efficient
Fig. 5.17 Effect of addition of different phosphorus sources on effluent decolourization (1-BHM, 2-Na$_2$HPO$_4$+NaH$_2$PO$_4$, 3-K$_2$HPO$_4$+KH$_2$PO$_4$).

Fig. 5.18 Effect of varying glucose concentration on effluent decolourization.
Fig. 5.19 Effect of varying concentrations of sodium nitrate on effluent decolourization.

Fig. 5.20 Effect of varying phosphate concentration on effluent decolourization.
B. *pumilus* sp. SRS83 under above optimized nutritional and cultural conditions. A 87.18% and 84.91% reduction in COD and ADMI values, respectively of the effluent having an initial COD and ADMI value 5632 and 2674 respectively was noticed (Photoplate 5.2) was noticed after 48 h upon treatment by *B. pumilus* SRS82 suggesting its efficiency in industrial applications.

Photoplate5.2 Decolourization of textile industry effluent (control) dye by *B. pumilus* SRS83.
5.4 Conclusion

The results, thus, obtained have characterized and identified dye degrading efficiency of *B. pumilus* SRS83. Static anoxic condition was found favourable for decolourization of AB210 by *B. pumilus* SRS83. The ability of the strain to tolerate, decolourize and degrade azo dyes at varying salt, pH, temperature and dye concentration; as high as 2500 mg/L, gives it an advantage for treatment of textile industry wastewater. Kinetic study of the decolourization experiments, indicate first-order reaction kinetics with respect to temperature and dye concentration. Enhanced AB210 decolourization ability by *B. pumilus* SRS83 could be due to the selection of efficient strain and optimization of the process parameters. Degradation of dye AB210 could be strongly attributable to the synergistic effect of enzymes such as, azoreductase, laccase, tyrosinase and NADH-DCIP reductase secreted by the bacteria. The formation of amines in the microaerophilic stage and their disappearance in the aerobic stage was confirmed by the FTIR analysis. The use of a single microorganism in a sequential static-shaking process was found to be very effective in the azo dye decolourization. Furthermore, microbial and phytotoxicity studies suggested that the degradation products of AB210 were much less toxic to the agriculturally important bacteria and crops. *B. pumilus* SRS83 proved to be highly promising microorganisms both for the application in the treatment of dyeing wastewater and in bioremediation of recalcitrant AB210 and other azo dyes.