Pseudomonas aeruginosa and Ochrobacterium anthropi isolated from the soil and Flavobacterium multivorum, Pseudomonas fluorescens and Pseudomonas putida have been examined for their ability to degrade different concentrations of Navitan Fast Blue S5R in the mineral salt media containing glucose and ammonium nitrate. It has been observed that Pseudomonas aeruginosa was the best degrader, able to degrade upto 1200 mg l\(^{-1}\) followed by Flavobacterium multivorum, which could degrade upto 1000 mg l\(^{-1}\). Pseudomonas aeruginosa was chosen as a possible candidate for azo dye decolorization in this investigation. The various environmental and nutritional factors influencing dye decolorization were studied to maximize dye decolorization.

Dye decolorization was influenced by the growth pattern of the bacterium and decolorization started when the organism reached late exponential growth phase. Studies on the time course of decolorization of Navitan Fast Blue S5R by Pseudomonas aeruginosa at different concentrations of dye upto 1500 mg l\(^{-1}\) in mineral salt medium with glucose and ammonium nitrate have shown that the organism decolorized the dye upto the concentration of 1200 mg l\(^{-1}\) and the initial rate of dye decolorization was in the range of 46-80% at 8 h and reached nearly 75% by 24 h, irrespective of the concentration of the dye studied. After 48 h, the dye was completely decolorized upto 200 mg l\(^{-1}\) but above this concentration there was not much change in the decolorization levels.

The bacterium was found to have appreciable level of growth at the pH range of 7-10 with pH 7.0 being the optimum for maximal dye decolorization.
The optimum temperature was found to be 30-35°C. Bacterial growth was influenced by the pH of the medium and the temperature of incubation and maximum dye decolorization was found to be mostly associated with maximal growth.

Preliminary studies have revealed that the dye could not be used as the sole carbon and nitrogen source by the bacterium and the organism required addition of carbon and nitrogen sources to co-metabolize the dye. Glucose was the best carbon source for growth and dye decolorization followed by arabinose and xylose. The dye decolorization was maximal at 0.5% glucose concentration. Increasing the concentration of glucose above this level did not have much effect on both growth and dye decolorization.

Of the different organic and inorganic nitrogen sources, ammonium nitrate was found to be the best nitrogen source supporting both growth and dye decolorization. Organic nitrogen sources supported the growth of the organism but decolorization was found to be at the lower levels. The organism required a minimum of 0.05% ammonium nitrate for dye decolorization. There was a greater increase in cell growth and dye decolorization, when both organic and inorganic nitrogen sources were added together at 0.1 and 0.05% w/v, respectively.

The dye was almost completely decolorized under both shake and static culture conditions but the rate of decolorization under shaken culture condition was slightly faster. Moreover, the aromatic amines formed were further degraded completely only under shaken culture conditions and CO₂
production was 10 fold higher than that under still culture conditions, indicating that O$_2$ is needed for the complete decolorization of the dye by *Pseudomonas aeruginosa*.

Isolation and identification of intermediate products formed during Navitan Fast Blue S5R decolorization by *Pseudomonas aeruginosa* were done by analyzing the culture supernatant at different time intervals. Analysis by various spectroscopic and chromatographic techniques revealed the presence of metanilic and peri acid as degradation products of Navitan Fast Blue S5R indicating that the first step in dye degradation was reduction of azo bond. HPLC analysis showed that the amines formed were further degraded under shake culture condition and were accumulated under still culture condition.

HPLC analysis of ethyl acetate extract revealed the presence of aniline, the desulfonated product of metanilic acid. The presence of aniline was also proved by GC-MS analysis. The presence of β-ketoadipic acid identified from the GC-MS indicates that aniline formed might have undergone oxidative deamination to catechol which through the ortho pathway reaction involving hydroxylation and decarboxylation reactions resulted in the formation of β-ketoadipic acid which in turn enter TCA cycle ultimately resulting in the release of aniline carbon as CO$_2$. HPLC analysis also confirmed the presence of salicyclic acid, which must have come from the naphthalene part of the dye. Based on these findings probable pathway for the degradation of Navitan Fast Blue S5R was proposed.
Degradation of aromatic amines namely metanilic acid and peri acid, the intermediate products formed during the degradation of Navitan Fast Blue S5R by Pseudomonas aeruginosa was also studied. The organism was able to degrade 90% of metanilic acid upto the concentration of 500 mg l\(^{-1}\) whereas only 25% was degraded when the concentration was 1500 mg l\(^{-1}\) and growth was found to be extensive in the presence of metanilic acid. The organism could utilize metanilic acid as carbon source and brought out nearly 65% degradation in the absence of glucose but required ammonium nitrate for degradation. When both glucose and ammonium nitrate were present complete degradation of metanilic acid was observed. Studies on the growth kinetics of Pseudomonas aeruginosa in the presence of metanilic acid has shown that metanilic acid degradation started when the organism reached its logarithmic phase and degradation increased with increasing incubation period and nearly 89% of metanilic acid was degraded after 24 h at 100 mg l\(^{-1}\).

HPLC analysis confirmed the degradation of metanilic acid and IR analysis of the spot obtained from TLC plates indicated the presence of an aliphatic acid. GC-MS analysis confirmed the formation of aniline and \(\beta\)-ketoadipic acid, which showed that aniline might have undergone degradation through ortho pathway resulting in the compounds that enter TCA cycle.

Studies on the biodegradation of peri acid by Pseudomonas aeruginosa showed that the organism was able to degrade 85% of the peri acid when the concentration of peri acid was 100 mg l\(^{-1}\) and the efficiency of Pseudomonas aeruginosa decreased as the concentration of peri acid was increased and at
1200 mg l\(^{-1}\) only 29.2% of the peri acid was degraded. Similar to the observations made in the case of metanilic acid degradation, the organism could utilize peri acid as carbon source and was able to degrade nearly 70% in the absence of glucose whereas, in the absence of ammonium nitrate only 8.2% peri acid was degraded. In the presence of both glucose and ammonium nitrate nearly 85% of peri acid was degraded. Kinetic study also shows that the resting cells of *Pseudomonas aeruginosa* are actively involved in the mineralization of peri acid and degradation started and accelerated when the organism reached the late exponential growth phase.

HPLC analysis confirmed the degradation of peri acid and also indicated the formation of salicylic acid. GC-MS analysis indicated the presence of phthalate that might have come from naphthalene part of the amine. Further, IR analysis of the spot obtained from TLC plates provided evidence for the presence of aliphatic acid.

Nearly 53% of carbon and 57% of nitrogen present in metanilic acid were converted to CO\(_2\) and NH\(_3\) whereas, 55% of carbon and 64% of nitrogen present in peri acid were converted to CO\(_2\) and NH\(_3\) during the course of degradation of the corresponding amines by *Pseudomonas aeruginosa*.

Azoreductase, the enzyme catalyzing the reduction of azo bond (-N=N-) has been isolated from the bacterial cells and purified by precipitation with ammonium sulphate followed by anion exchange chromatography on DEAE cellulose and size exclusion chromatography by Sephadex G-100. The
specific activity was significantly increased and 53 fold purification of the enzyme was achieved.

The results obtained on the time, substrate and enzyme concentration activity relationship indicate that Beer's law is followed upto about 30 min of enzyme substrate reaction when the substrate and enzyme concentration do not exceed 100 µm and 500 µg, respectively.

The purified enzyme was found to be active over a pH range of 6-8 with the maximum activity at pH 7.0. The enzyme showed 48% activity at pH 9.0 and was found to be quite stable in the pH range of 6-8. The enzyme was found to have maximum activity at 35°C and was stable upto the temperature of 35°C.

A study on the influence of different metal ions on azoreductase activity revealed that except Mg²⁺, which has a slight positive effect none of the metal ions enhanced the azoreductase activity. The enzyme activity was unaffected by the presence of Ca⁺. The activity was inhibited by Cu²⁺, Fe²⁺ and Zn²⁺ ions. The molecular weight of Pseudomonas aeruginosa was determined to be 29,000 by gel filtration method. SDS-Polyacrylamide gel electrophoretic study of the enzyme revealed a single band and its apparent molecular weight was calculated to be 28,000. Addition of NADH enhanced the enzyme activity and the enzyme utilized NADH as a source of electron donor for reduction of azo group in the dye during decolorization reaction. The Km value of azoreductase using Navitan Fast Blue S5R as substrate was determined to be 0.178 mM using the Lineweaver – Burk plot. The enzyme
was able to degrade different azo dyes and the Km values differed with the nature of the azo dyes. The results also show that Navitan Fast Blue S5R has more affinity towards the enzyme followed by Amaranth (Km 0.285 mM), Atul Acid Black (Km 0.33 mM) and were readily degraded by azoreductase.

*Pseudomonas aeruginosa* is found to be highly efficient in degrading different azo dyes in tannery effluent under both shake and still culture conditions. The present investigation also shows that the bacterium could degrade mixture of dyes of different chemical nature. The bacterium could also degrade the aromatic amines (metanilic acid and peri acid) when present in the untreated effluent. It is apparent from the research finding that *Pseudomonas aeruginosa* can co-exist along with the native organisms of the effluent but it appears to be a potent microorganism compared to the bacterial populations present in the effluent.

The treatment of dye laden waste water by purely biological processes including *Pseudomonas aeruginosa* in the system may be possible even without the inclusion of external carbon and nitrogen sources as it is evident from the results. This may be the fact that the bacterium might utilize the carbon sources derived from partially digested lignin and cellulose based materials from sources like cow dung and nitrogen sources coming from proteins and aminoacids from the raw materials hides/skins and ammonium salts from the deliming operations of leather processing discharged into the effluent.
This investigation also revealed the fact that indigenous bacteria of the effluent also decolorizes the azo dyes to certain extent but to a lesser extent compared to *Pseudomonas aeruginosa*. It is also evident that though these native populations may decolorize the dye by attacking the azo group further degradation of the more toxic amines are limited. Introducing *Pseudomonas aeruginosa* to the effluent would ultimately result in the more complete and faster biodegradation of the intermediate products of azo dye decolorization which most organisms normally do not.

The findings of these present research investigations identify an unique bacterial system *Pseudomonas aeruginosa* capable of decolorizing various kinds of azo dyes and degrading their intermediate products, the arylamines from the tannery effluents.