5. Discussion

Water represents the medium of life on earth and one of our four ancient elements. It is a resource which cannot be produced or added as when required by any technological means. The impurity or contamination of natural water which results in the alteration of its physical, chemical or biological properties which is called as water pollution. Industries are the main source of polluting water bodies. They are characterized depending upon their productive types and pollution load. (UNEP/IEO 1999). Industries such as textiles, engineering, electronics and tannery etc. are flourishing in recent years (Islam et. al., 1997). Textile industries are one of the largest water users and polluters resulting in high wastewater generation (Nemerow 1978, Ghoreishi and Haghighi 2003). Textile effluents offer the alteration of physical, chemical and biological properties of aquatic Environment.

5.1 TEXTILE INDUSTRIES:

Textile and clothing is one of the largest, oldest and fastest developing sectors in India. The type of dyes and chemicals used in the textile industry are found to differ depending on the fabrics manufactured. This industry is particularly known for its high water consumption as well as the variety and amount of chemicals used throughout different process stages. The Environmental problems associated with textile effluents are partially due to high inorganic salt and alkali content. The amount of water consumed and released also varies depending on the type of fabrics produced. About 0.08-0.15 cubic meter of water is used to produce 1 kg of fabrics Pagga and Brown (1986) Al-Kdasi et. al., (2004) and Moustafa, (2008), who estimated that about 1,000- 3,000 m3 of water is let out after processing about 12-20 tonnes of textiles per day. The biorefactory nature of textile waste water from the dyeing and finishing stage is mainly attributed to the extensive use of various dye stuffs (Arslan et al., 1999). Dyeing and finishing are known to be very important steps in textile manufacturing processing terms of Environmental
concern (Ince and Gonenc 1996). A strong colour of waste water effluent, if not treated would cause problems to ecological systems of the receiving waters (Lin and Chen 1997). The colour of textile wastewater is mainly due to the presence of textile dyes, pigments and other coloured compounds. A single dyeing operation can use a number of dyes from different chemical classes resulting in a complex wastewater (Correia et al., 1994). Moreover, the textile dyes have complex structures, synthetic origin and recalcitrant nature, which makes them obligatory to be removed from industrial effluents before being disposed into hydrological systems $(Anjaneyulu et al., 2005)$. Untreated textile waste waters are increasingly affecting plant soil and thus polluting the water bodies. These effluents are rich in dyes and chemicals, some of which are non-biodegradable and carcinogenic and pose a major threat to health and the Environment if not properly treated.

In the present study the textile dyeing effluent was collected from Erode near Bhavani and the physico-chemical characteristics were analyzed following the standard methods (APHA 1998). The characteristics of textile dyeing effluent is presented in the Table No: 1. It was inferred from the table that the effluent had a characteristic intense colour with an alkaline pH, high Total dissolved solids, Chemical oxygen demand, Biological oxygen demand, Chlorides, Total hardness, calcium, Magnesium, sulphate, Nitrogen. The observation coincides with work of Patnaik et al., (1996), Deo and wasif (1999), Shenai (2001), Teli (2001) and Loshi (2004). Effluent from textile industries are a complex mixture of many polluting substances such as heavy metals, pigments and dyes. Among the various types of organics present, colour is the most difficult one to remove. colour indicates an increased BOD and COD (Tripathy, 2007). The effluents have strong concentration of COD, phenol, and its derivatives and often contains proteins, cyanides, chlorinated lignin compounds and dyes and heavy metals like chromium, copper and nickel etc (Borja et al., 1992). Without a proper treatment, the
discharge of textile dyeing effluent to the Environment may cause serious and longstanding consequence (Nicolaou and Hadjivasillis 1992). The treatment of textile dyeing effluent requires the use of efficient treatment method to meet the direct discharge standards (Tunay et al., 1990; Joshi, 2004; Sharma 2005).

5.2 TYPES OF TREATMENT

The chemical treatment methods include adsorption, coagulation-flocculation with inorganic coagulants and organic polymers; chemical oxidation; ozonation; electrochemical process, ion exchange, chemical precipitation, reverse osmosis and evaporative recovery (Stasinakis 2008). Although all these techniques are very versatile and useful, they all end up in producing a secondary waste product which needs to be tackled further. (Rafu and Salman 2009, Preethi Mehta et al., 2012). Among the biological techniques, bioremediation has evolved the most promising one because of its economical, safety and Environmental features, since organic contaminants become actually transformed and some of them are fully mineralized (Susana Saval, 2003). Besides, conventional methods for treatment of toxic chromate (Ohtake and Silver, 1994) required large amount of chemicals, energy and are unsuitable for small scale leather dye and electroplating units.

Hence suitable method for the treatment of textile industry effluent must be identified.

5.2.1 Chemical treatment of the effluent

Chemical treatment represents the conversion or transformation of pollutants by chemical oxidation agents other than oxygen or bacteria. Chemical methods include coagulation or flocculation combined with flotation and filtration, precipitation-flocculation, electro-flotation, electro-kinetic coagulation, conventional oxidation methods by oxidizing agents, irradiation or electrochemical processes (Sharma, 2005).
Coagulation method is one of the most used methods, especially in the conventional treatment process. In our present study, the coagulants such as sodium bicarbonate, ferrous sulphate and titanium dioxide are used. Added coagulants cause the solids to settle. In the study of (Ng et al., 1987), four types of coagulants such as alum, ferrous sulphate, polyelectrolytes and lime were used. Arabindo et al., (1998) used ferrous sulphate as a coagulant.

In the present study, the effluent was chemically treated with different concentration of chemicals such as ferrous sulphate, sodium carbonate and titanium oxide, ranging from 100 to 1000 ppm. The alkalinity value was reduced to 194 mg/L by titanium oxide at 700 ppm. It is represented in Table No: 2. whereas sodium bicarbonate and ferrous sulphate reduces the value to 200 and 238 mg/L. The TS, TDS, calcium, hardness, COD and Dissolved oxygen content was also greatly reduced. The parameters such as acidity, hardness, nitrogen, sulphate and chloride value also reduced to considerable extent. This was in accordance with the results of Bulusu et al., (1968), Mahyabhat et al., (1988), Tunay (1990), Petr Grau and Przepiora et al., (1998). Baskaran and chakravarthy (1973) reported that by use of different types of coagulants, the suspended solids and BOD can be reduced to a significant level.

Among the three chemicals the titanium oxide is efficient in treating the effluent. This results coincides with the study of Al-sayyed et al., (2001) where oxidation of titanium oxide is suggested to have a larger advantage over oxidation of hydrogen peroxide this is because titanium dioxide is found to absorb light up to 385 nm.

The main disadvantages of this treatment are the process control that is a little difficult, the potential affection of precipitation rate and floc size by impurities. The sludge production which has to be settled dewatered and pressed into a cake for subsequent landfilling tipping. Ghaly et al., (2014).
5.2.2 Biological treatment.

Biological treatment can be defined as the decomposition or destruction of contaminant molecules by the action of microbes or enzymatic machinery of a biological system. Biological method of degradation can be done by microbes like bacteria, fungi and algae. This could be a viable option as a low–cost an eco-friendly decentralized waste water treatment. (Pearce et al., 2003, Forgacs et al., 2004, Santos et al., 2007, Kaushik and malik 2009).This method of effluent treatment have the advantage of being cost-effective and in addition to colour removal, they can also reduce both the biological oxygen demand (BOD) and chemical oxygen demand (COD) of the waste water (Eaton,1980) . The biological process removes dissolved matter in a way similar to the self -depuration but in a further and more efficient way than chemical method. The removal efficiency depends upon the ratio between organic load and the bio mass present in the oxidation tank, its temperature, and oxygen concentration.

In our present work, the microbes such as bacterial and fungal isolates were selected for the treatment of textile effluent. Aerobic decolourisation of azo dyes by bacteria as well as fungal cultures were reported by many authors. (Dykes et al., 1994., Sumathi and Manju 2000). Anaerobic biological treatment process makes use of the anaerobic bacteria that decompose organic matter in anaerobic conditions. This method was first used for sludge digestion. A major advantage of anaerobic system along with effluent decolourization is the production of biogas, reusable for heat and power generation that will reduce energy costs.

The accumulation of chemicals and dyes by microbial mass has been termed biosorption. (Hu ,1992 ).The other biological treatment method includes bioaccumulation was defined as accumulation of pollutants by actively growing cells by metabolism and temperature independent and metabolism dependent mechanism steps (Nigam et al.,2000).One method for removing the undesirable compounds from the Environment is bioremediation –application of
biological treatment to clean up hazardous chemicals. The ability of microbes to degrade a vast array of pollutants makes bioremediation a technology that can applied in different soil conditions. Bioremediation technologies include myco remediation, phyto remediation, bioventing, bioleaching, land framing, bioreactor, composting, bio augmentation, rhizo filtration, and biostimulation.

Bioremediation constitutes the use of natural biota and their processes for pollution reduction. Bioremediation is an integrated management of polluted Ecosystem where different microorganisms are employed which catalyze the natural processes in the polluted or in the contaminated aquatic or terrestrial Ecosystem. In bioremediation process the usage of the bacteria, fungi and enzymes to degrade Environmental pollutants is carried out. Bioremediation is a natural process and is therefore perceived by the public as an acceptable waste treatment process for contaminated material such as soil or water. The residues after the treatment are usually harmless products and include carbon dioxide, water and cell biomass. Theoretically, bioremediation is useful for the complete destruction of a wide variety of contaminants. This eliminates the chance of future liability associated with treatment and disposal of contaminated material. Instead of transferring contaminants from one Environmental medium to another, for example, from land to water or air, the complete destruction of target pollutants is possible by using bacteria. This also eliminates the need to transport quantities of waste off site and the potential threats to human health and the Environment that can arise during transportation. Bioremediation can prove less expensive than other technologies that are used for clean-up of hazardous waste (Vidali, 2001).

The advantages of biological technologies for the removal of pollutants, first biological processes can be carried out in situ at the contaminated site, second bioprocess technologies are usually Environmentally benign and third exsitu method are cost effective. There are wide variety of microorganisms capable of decolourising wide range of dyes. Decolourisation of dyes
with pure culture was impractical, as the isolated culture would be dye specific (Vijaraghavan and Yeoung-sang Yun, 2008). In the present study the bioremediation of pollutants and heavy metals in textile effluent is carried out using bacterial and fungal microorganisms.

**5.3 ISOLATION OF MICROORGANISMS FROM TEXTILE EFFLUENT.**

Bacterial and fungal colonies were isolated from the textile dyeing effluent. In our present study, the bacteria such as *Pseudomonas flurosence*, *Bacillus subtilis*, *Bacillus amyloliquifaciens*, and *Escherchia coli* were isolated. It is given in the Table No: 4 and **Plate No: 4.** Among the different bacterial isolates, *Pseudomonas flurosence* and *Bacillus subtilis* were found to be predominant form. *Bacillus amyloliquifaciens* and *Escherichia coli* were found to be sub dominant form. The result correlates with the studies of Ilakiya et al., (2011), were the dominant bacterial strains are *Pesudomonas aeroginosa* and *Bacillus lichiniformis*. Also in the studies of Saravana Babu et al., (2010), the predominant bacterial strains such as *Pseudomonas putida* and *Bacillus subtilis* were isolated from the tannery effluent. A number of bacterial species belonging to genera *Bacillus*, *Micrococcus*, *Proteus*, *Pseudomonas*, *Sphingomonas* and *Staphylococcus* were reported to have been isolated for bio-treatment of textile dyes (Ali et al., 2009; Zhang et al., 2010; Ayed et al., 2011). Chen et al., (2011) reported that bacterial strains that are able to degrade aromatic hydrocarbons have been repeatedly isolated, mainly from soil belonging to the genus *Pseudomonas*. The biodegradative pathways have also been reported in bacteria from the genera *Mycobacterium*, *Corynebacterium*, *Aeromonas*, *Rhodococcus* and *Bacillus* (Morzik et al., 2003).

In our work, the fungal isolates such as *Aspergillus niger*, *Aspergillus fumigatus* and *Alternaria alternate* were isolated from the textile effluent. It is represented in the Table No: 6 and **Plate No: 5.** Results coincides with the study of Brodkorb and Legge (1992) and Heinfling et al.,(1997), were the 36 isolated fungal strains are tested for their ability to
decolourise the dye effluent. A newly screened *Aspergillus fumigates* XC6 examined for its ability to decolourise a dye industry effluent (Xian-chun jiin *et al.*, 2006). The fungi such as *Aspergillus ochraceus*, *Aspergillus terreus*, *Aspergillus niger*, *Penicillum citrinum* and *Fusarium moniliforme* were isolated from dye effluents (Muthezhilan *et al.*, 2008).

### 5.4 BIOREMEDIATION USING BACTERIA AND ITS ENZYME

The prokaryotes especially bacteria, are the most important organisms for bioremediation. Bacterial biodegradation of textile dyes is an attractive and inexpensive method. Isolation of new strains or adaptation of existing strain for decomposition of dyes will increase the efficiency of bioremediation. Most of the studies on azo dyes biodegradation have focused on bacteria and reported the decolourisation of effluent from the textile industry by *Pseudomonas* and *Bacillus* (Lin 1992, Yatome 1993). In our present study the bacterial isolates such as *Pseudomonas flurosence*, *Bacillus subtilis*, *Bacillus amyloliquifaciens* and *Escherchia coli* were used to treat the effluent. Similar isolates such as *Bacillus subtilis*, *Bacillus cereus*, *Pseudomonas* species, were used in the study of Chang *et al.*, (2001).

In the present study, the bacterial enzymes such as manganese peroxidase, lignin oxidase was extracted from the bacteria *Pseudomonas flurosence* and *Bacillus subtilis*. This findings correlates with the work of Husain (2009), were the bacteria, white-rot fungi, mixed microbial cultures from a wide variety of habitats are found to be able to degrade dyes using enzymes, such as lignin peroxidases (LiP), manganese dependent peroxidases (MnP), H2O2-producing enzyme such as glucose-1-oxidase and glucose-2-oxidase, along with laccase, and a phenoloxidase enzyme.

### 5.5 TREATMENT OF EFFLUENT WITH BACTERIA AND ITS ENZYMES

The physico-chemical parameter of the effluent was analysed after the incubation period of 10 days. It was found that all the parameters was reduced by the treatment with bacteria.
The pH of the effluent was 9 which was slightly alkaline when compared to the acidic pH of the dyeing effluent in one of the studies of Tyagi and Mehra (1990). The pH of the effluent alters the physico-chemical properties of water which in turn adversely affects aquatic life, plant and humans. On treatment with bacterial isolates, the pH was reduced to 8.5 by *Pseudomonas flurosence*. In the study of Smrithi and Usha (2012), the pH was reduced to 7.8 on treatment with *Bacillus*. It is given in the Table No: 8.

The TDS values of the effluent sample was high than the permissible limits but when compared to a textile effluent collected from a mill near Haryana was found to be low. (Senthilnathan and Azzez 1999). The *Pseudomonas flurosence* reduces the TDS value to 3700 mg/L. It is given in the Table No: 8. In the studies of Arun Prasad and Kokati Venkata Bhaskara Rao (2011) the TDS value reduced to 5755 from the raw effluent value of 6025 mg/L.

The DO level of the treated effluent reduced to 1.9 mg/L by *Bacillus* from the raw effluent value of 2.9. It is represented in the Table No: 8. This correlates with the study of Mahmood *et al.*, (2013), where the DO level get reduced to 2mg/L on treatment with *Pseudomonas*. The values of acidity, ALKALINITY, hardness, chloride, nitrogen, BOD, COD and sulphate was also reduced significantly by *Pseudomonas* than the other isolates. Similar trend was observed in the studies of Ajao *et al.*, (2011), where the *Pseudomonas aeruginosa* reduce the value of COD, BOD, sulphate, DS considerably than the *Bacillus subtilis*. The raw effluent exhibited high value of heavy metal chromium and Nickel which was of the same order of magnitude reported in the textile effluent sample reported by Kim (1994). The Chromium content was reduced to 0.36 mg/L by *Pseudomonas* than the *Bacillus*.

In our study on effluent treatment with bacterial isolates showed that, the physico-chemical parameters such as alkalinity, TDS, calcium and COD was significantly reduced.
Comparing the bacterial isolates, *Pseudomonas* efficiently reduces all the physico-chemical parameters than *Bacillus* and *Escherchia coli*. Depali (2011) reported that the physico-chemical parameters was reduced greatly by *Pseudomonas putida* than *Bacillus* sp.

In the bacterial enzymatic treatment, the enzyme extracts of bacterial isolates were treated to the effluent. All the physico-chemical parameters were reduced on treatment with bacterial enzymes. Among the physico-chemical parameter, TS, TDS, Calcium, Hardness and COD were greatly reduced. Among the bacterial enzymes, the enzymatic extract of *Pseudomonas* and *Bacillus* are effective one in treating the effluent. Similar results were obtained in the study of the biochemical purification, characterization of chromate reducing flavoenzyme from *Pseudomonas putida* was identified (Park *et al.*, 2000 and Ackerley *et al.*, 2004). The treatment of effluent with chromate reducing flavoenzyme from *Pseudomonas ambigua* had been characterized (Suzuki *et al.*, 1992).

In the present study, bacterial treatment showed that, *Pseudomonas fluroscence* was efficient in reducing the Physico-chemical parameter of the effluent.

### 5.6 BIOREMEDIATION OF FUNGI AND ITS ENZYME

Fungi are involved in the biodegradation of undesirable materials or compounds and convert them into harmLess, tolerable or useful products. Many organisms are involved in the biodegradation of organic waste, which has resulted in the biodegradation of organic waste, which has resulted in the production of novel substances of biotechnological importance. White rot fungi, are however efficient in the biodegradation of recalcitrant compounds like xenobiotics, lignin and dye stuffs by their extracellular ligninolytic enzyme system (Brodkorb and Legge, 1992).

In the present study, the fungi such as *Aspergillus niger*, *Aspergillus fumigatus* and *Alternaria* were isolated from textile effluent. From the effluent treatment with fungi, it is
inferred that all the physico-chemical parameters were efficiently reduced. Among the different forms *Aspergillus niger* was found to be dominant one. *Aspergillus niger* was found to be efficient in reducing the physico-chemical parameters such as acidity, ALKALINITY, total dissolved solids, chemical oxygen demand, biological oxygen demand, hardness, magnesium, calcium, chloride, sulphate. Similar results also observed by Narandar and Sivasamy (1988). Heterotrophic fungi *Mucor, Aspergillus, Penicillium* and *Yarrowta*, remove both soluble and insoluble metal species from solution and are also able to leach metal cations from solid waste White *et al.*, (1997). *Aspergillus niger* grew profusely on tan liquor but grew ascantily on effluents from composite liquor and effluent after filtration. Similar work was done by Xian-Chun Jin *et al.*, (2006) reported that *Aspergillus niger* isolated from mildwing rice straw was evaluated for its ability to decolourize a dye industry effluent. *Aspergillus niger* is an excellent strain for the decolourisation of reactive textile dyes effluents, and that it might be a practical alternative in dyeing waste water treatment. Sharma *et al.*, (2003) reported that 32 fungal species were effective in dye decolourisation.

Fungi are recognized for their superior aptitudes to produce a large variety of extra cellular enzymes, organic acids and other metabolites and for their capacities to adapt to severe Environmental constrains (Lilly and Barnett, 1951; Cochrane 1958). Fungal systems appear to be the most appropriate in the treatment of coloured and metallic effluents was reported by Ezeronye and Okerentugba. (1999).

In the present study, the enzymes such as laccase and peroxidase was extracted from fungus *Aspergillus niger* and *Aspergillus fumigatus*. The enzymes were used to treat the effluent. In the enzymatic treatment all the parameters were reduced. Among the physico-chemical parameter, TS, TDS, Hardness, Calcium value were greatly reduced. Among the enzymes the enzymatic extract of *Aspergillus niger* was efficient in reducing the physic-chemical parameters.
This coincides with the work of Gozezynski et al., (1994) and Spadaro et al., (1992) were the role of peroxidase enzymes from *Aspergillus niger* in degradation of dyes has been documented. The extracellular enzyme system also enables white rot fungi to tolerate high concentration of pollutants (Knapp et al., 1997). Laccase has been regarded to be Environmentally friendly and it is considered to be an attractive option for the development of new methodologies in treating the textile effluents. Laccase, are a small group of enzymes denominated as ‘blue oxidases’. Fungal treatment of effluents had a tendency to become very protracted, immobilized enzyme treatment may hold a better potential for dye decolourisation and recycling of effluents without the need for the addition of growth substrates. Direct enzyme treatment was more efficient than the traditional physical chemical treatments and microbial culture method. Abadulla et al., (2000) have assessed the potential of *Trametes hirstua* and a laccase from this organism to continuously degrade textile dyeing could be reduced by enzyme remediation with laccases. Two peroxidases lignin and Mn peroxidase produced by *Phanerochaete chryosporium* and laccase from *Pyricularia oryzae* appear to initiate azo dye degradation. (Chivukula and Reganathan 1995, Cripps et al., 1990, Goszynski et al., 1994, Pasti-Grigsby et al., 1992, Spadaro et al., 1992, Spadaro and Renganathan 1994).

### 5.7 COMPARISON OF BACTERIAL AND FUNGAL TREATMENT.

Among the isolated bacterial strains such as *Pseudomonas fluroscence, Bacillus subtilis, Escherchia coli* and *Bacillus amyloiqificiens*, the *Pseudomonas fluroscence* was found to be an effective in reducing the physico-chemical parameters of the effluent. Among the fungal strains, *Aspergillus niger, Aspergillus fumigatus, Alternaria alternata*, the *Aspergillus niger* was effective. The Physico-chemical parameters such as alkalinity, DS, calcium and COD values are considerably reduced by bacterial isolate than the fungal isolate. The alkalinity value was reduced to 162 mg/L from the value of 395 mg/L by *Pseudomonas fluroscence*, whereas the
fungi, *Aspergillus niger* reduces the alkalinity value to 170 mg/L. Similarly, the TDS value was reduced to 3700 mg/L from the value of 6800 mg/L by bacterial isolates. Whereas the fungal isolates reduces the value to 4120 mg/L. The bacteria have capability to grow fast in polluted Environment, required minimal nutrition and rapid growth can be used to achieve good results in decolourisation experiments than the fungi. (Greenberg *et al.*, 1992). Interesting in aspects the bioremediation of pollutants using bacteria has intensified in recent years, as many researches demonstrated the efficacy of bacterial bioremediation over fungal isolated and actinomycetes as reported by Arun Prasad and Kokati Venkata Bhaskara Rao (2011). Comparatively among the bacterial and fungal treatment of the effluent, the bacterial isolate *Pseudomonas flurosence* is more effective in reducing the physico-chemical parameter of the textile effluent than the fungal isolates.

### 5.8 GENETICALLY TRANSFORMED BACTERIAL TREATMENT.

The bacterial isolates such as *Pseudomonas flurosence, Bacillus subtilis, Bacillus amyloliqifaciens* and *Escherchia coli* were genetically transformed. The Physico-chemical parameters were significantly reduced on treatment with transformed isolates than the normal forms. The ALKALINITY value get reduced to 120 mg/L by transformed *Pseudomonas* than the normal isolate of *Pseudomonas flurosence*. The TDS value get reduced to 2100 mg/L by transformed *Pseudomonas* which is three times efficient than the normal forms from the value of 6800 mg/L. The calcium value was also reduced to 130 mg/L by transformed *Pseudomonas flurosence* than the normal form. Similarly the COD get reduced to 20 mg/L, by transformed *Pseudomonas flurosence* than the normal isolate value of 29 mg/L. The other physico-chemical parameters were also greatly reduced by the treatment with transformed isolates when compared to the normal form. Among the transformed isolates, the *Pseudomonas flurosence* was considered as an efficient one in reducing the physico-chemical
parameter. This correlates with the study of (Keyhan et al., 2004) were the chromate toxicity was reduced by the transformed molecular engineering cells of Pseudomonas flurosence and enzymes to decrease chromate toxicity and increase the level of chromate transformation. The biochemical purification, cloning and characterization of Chr R, a class I chromate reducing flavoenzyme from Pseudomonas putida was reported by Park et al., (2000) and Ackerley et al., (2004).

The genetic studies of Transformed bacterial forms shows that all the physico-chemical parameters was efficiently reduced by the transformed bacterial forms than the normal isolates. The transformed Pseudomonas flurosence was efficient than the transformed Bacillus subtilis. Similar results obtained in the study of Sukumar et al., (2006) were the mutated strains of Bacillus grew faster and reduced the colour of the dye effluent comparatively greater than the wild isolate. In the study of Vivek kumar chaudhary and Debajit Borah (2011), the transformed mutant of Pseudomonas has a greater potential to degrade hydrocarbon when grown in a medium containing used engine oil than the mutated strains of Bacillus and Corynebacterium.

5.9 COLOUR REMOVAL USING BIADSORBENTS.

Colour is aesthetically objectionable and it also reduces light penetration into water decreasing the efficiency of photosynthesis in aquatic plants, there by having adverse impact on their growth (Bhattacharya and Sarma, 1997). In the present study, for the decolourisation of the effluent, the bioadsorbents such as neem leaf powder, sugarcane waste, and coir pith was selected to treat the effluent. In the study of Mane and Bhusari (2012), the feasibility of orange peel and banana peel were used as colour adsorbent capacity of material with initial concentration at different dose, time and pH. Khattri and Singh, (1998) reported that the neem sawdust have been used for the removal of dyes using different inorganic adsorbents, considered to be an effective one.
In the present study, optimization parameter such as effect of temperature, incubation period and particle size were considered for better colour removal. Sharmila and Muthusamy (2013) studied that biosorption studies were done using tea leaves as a function of various parameter such as effect of time, effect of dosage concentration, effect of particle size and effect of pH. The results of their studies show that the sorption percentage increased with increase of time up to 180 min. The percentage of lead removed increased with increase in spent tea leaves adsorbent size. It is also observed that the maximum adsorption percentage with the increase of mesh size up to 150.

On the treatment of effluent at different temperature the decolourisation was maximum at 45°C and at this particular temperature, sugarcane and coir pith removes the colour by 89 and 85% respectively. Whereas neem removed 91% of colour. In the study of Khattri and Singh (2000), the colour removal of dyes was studied using neem saw dust at different temperature ranging from 25 to 45 °C. It was found that at 45 °C the colour removal was 92%. The increase in temperature affects the solubility and chemical potential of the adsorbate, the latter being a controlling factor in adsorbtion. According to Gupta et al., (1990), the solubility of absorbate increases with the increase in temperature.

In the present study, on treatment of effluent at different incubation period such as 4 min, 8 min, 12min, 16min, and 20min the decolourization was maximum at 20min. In this time duration, the sugarcane, coir pith and neem removed 90%, 87% and 92% of colour respectively. The decolourisation percentage increased with the increase of time up to 180 minutes using tea leaves as a biosorbent in the study of Sharmila and Muthusamy (2013). The colour removal efficiency increases with an increase in contact time and this can be explained by the affinity of the adsorbents towards metal ions. (Ramana et al., 2002).
Among the different particle size, 100µm decolourized 90% of effluent by neem. The sugarcane waste and coir pith removed 85 and 84% respectively. The decolourisation increase with increase in particle size. In the study of Sharmila and Muthusamy (2013), the percentage of lead removal increased with increase in spent tea leaf adsorbent size. The maximum removal was obtained at 150 mesh size. As the particle size of the adsorbent decreased, the amount of dye adsorbancy increases over the saturation time. According to weber and Morris (1963), the breaking of larger particle tends to open tiny cracks and channels on the particle surface, providing added surface area.

Thus the overall decolourisation studies showed that the neem leaf powder has the capacity of maximum decolourisation ability than the sugarcane waste and coir pith. The maximum decolourisation was about 92% which occurs at 45 °C at 20 min with 100 mesh size. Similar result also obtained by Khattri and Singh (2000) were the neem saw dust was are found to be an effective one for the removal of basic dyes from aqueous solutions.

5.10 HEAVY METAL IN TEXTILE EFFLUENT

Heavy metal ion concentration is one of the major problem associated with the textile effluent, which depends upon the usage of higher amount of metal containing dyes in dyeing process (Correia et al., 1994; Sabour et al., 2001). The threat of heavy metal pollution to public health and wild life has led to an increased interest in developing systems that can remove or neutralize its toxic effects in soil, sediments and waste water. Heavy metals, even at low concentrations can cause toxicity to humans and other forms of life (Brierly et al., 1986). Removal of toxic metal ions from polluted effluents by using microbial biomass is being studied extensively by many people. (Akthar et al., 1996 Akhtar and Mohan 1995). Both live and inactivated microbial biomass of bacteria, fungi and algae have been utilized for removing toxic metal ions. (Kumar et al., 1992, Volesky 1994, Karna et al., 1996). The functional groups
involved in the binding of heavy metal to microbial cells are phosphates, carboxyl, hydroxyl groups. (Akthar et al., 2005, Karna et al., 1996). These biological metal removal process has distinct advantages over conventional methods. The process does not produce chemical sludges, could be highly selective, more efficient, easy to operate and hence, cost effective for the treatment of large volumes of waste waters containing low concentrations. (Deans and Dixon 1992). In the present work, the heavy metal such as chromium and nickel are selected for their removal from effluent.

5.10.1 Chromium

Hexavalent chromium is a wide spread Environmental pollutant, arising as a by-product of numerous industrial processes. Chromate is highly toxic, mutagenic and carcinogenic and because of its solubility in water, chromate reductase contamination can be difficult to contain (Singh et al., 1998). Bacteria can reduce Cr (VI) to Cr (III) offering promise for an Environmentally friendly and affordable solution to chromate pollution. Smrithi and Usha (2012), reported that the most toxic and carcinogenic hexavalent chromium is converted to minimal risk trivalent chromium by the Bacillus sp.

5.10.2 Nickel

Nickel is ubiquitous in nature. It occurs in the form of sulfide and silicate minerals. Nickel compounds are carcinogenic to human risk were highest for lung and cancer among workers heavily exposed to nickel sulfide, nickel oxide and ton metallic nickel. Blood nickel levels provide a guideline as to the severity of exposure (Krishna 2008).

In the present study, the removal of heavy metals such as chromium and nickel from textile effluent was also carried out.
5.11 RESISTANCE OF TRANSFORMED BACTERIAL ISOLATES TO HEAVY METALS

In the present study, resistance of the transformed bacterial isolates to heavy metal such as chromium and nickel was studied. The resistance was studied using plate method and well method. Nine different bacterial isolates were screened for the resistance to grew in 10 to 50 mg/L of Cr (VI) concentration. At 50 mg/L concentration the bacterial isolates showed maximum resistance. It was also reported in the study of Deepali (2011). In our study, the transformed *Pseudomonas* and *Bacillus* were studied for the heavy metal resistance. These transformed isolates showed maximum resistance up to 750 ppm concentration of Cr and Ni. It was found that the Zone of inhibition of heavy metal increases with increase in concentration of chromium up to 750 ppm. Above the concentration, the zone of inhibition gets decreased. This is because, the sorption increased with increase in concentration as long as binding sites were available. Also the microbial population in the system can affect chromium removal due to saturation of metal binding sites of the biosorbent. Cr binding was rapid initially, but also reaches capacity or equilibrium afterwards (Siegel *et al.*, 1986). The result showed that as the concentration of the metal increased the growth of bacteria decreases. This was in accordance with the work of Konopka *et al.*, (1999) were the microbial biomass generation was decreased as the concentration of heavy metal increased. According to Brown *et al.*, (1994), the microbes continued to be metabolically active in the presence of higher concentration of heavy metals but the number might be reduced. At the higher concentration, it induced toxicity and population reduction in microorganisms and it depends on the type of microorganisms and growth conditions of media. It was further supported by Hussein *et al.*, (2004) found that the amount of biomass production decreased while increasing the concentration of heavy metals.
5.12 REMOVAL OF HEAVY METALS FROM EFFLUENT

The raw effluent contains the chromium to about 0.41 mg/L. Nickel concentration of the effluent was 0.32 mg/L. The current sample exhibited value of heavy metals which was in accordance with same order of magnitude reported in textile effluent sample Kim (2005). In the study carried out by Malik and Ahmed (2002), the heavy metal such as Cd, Cu, Fe, Mn, Ni, Zn, K and Na were much higher than the permissible under the NEQS in the textile effluent. In the bacterial treatment of the effluent, the *Pseudomonas fluroscence* has removed the chromium to about 0.36 mg/L. The enzymatic treatment of *Pseudomonas fluroscence* remove 0.37 mg/L of chromium. In fungal treatment, 0.39 mg/L of chromium is removed by *Aspergillus niger*. Sayer *et al.*, (1997) reported that the *Aspergillus niger* is the most prevalent fungal strain in the waste water and they have bioremediation potential. Price *et al.*, (2001), found that similar capabilities of this strain, where *Aspergillus niger* remove 91% copper and 70% zinc from swine waste water. In the enzymatic treatment of fungi, *Aspergillus fumigatus* remove 0.40 mg/L of chromium. *Aspergillus terreus* and *Aspergillus niger* have demonstrated nickel uptake capability from aqueous solution (Dias *et al.*, 2002; Keshinkan *et al.*, 2004). The transformed *Bacillus subtilis* remove about 0.27 ppm of chromium and 0.20 ppm of nickel. In the studies of Kim (2005), the *Bacillus* sp. can grow in significant level of heavy metal and has ability to adsorb the Cu at a maximum level of 400 mg/L. It removes 65% of Cu during the active growth cycle. The transformed *Pseudomonas fluroscence* has removed chromium to 0.23 mg/L and nickel to 0.19 mg/L. In one of the studies, *Pseudomonas* was considered to be the most effective biosorbent because of its high adsorption capacity when compared to *Bacillus* and *Micrococcus* sp. *Pseudomonas* adsorbed 86.6% of Cd. (Hany Hussein *et al.*, 2004).

The overall removal of heavy metals showed that the transformed *Pseudomonas fluroscence* was efficient in the removal of both chromium and nickel. Most of
reviews reveal that *Pseudomonas* sp, is a suitable biosorbent to remove heavy metals like Cu, cd and Pb from aqueous solution. (Zaied *et al*., 2008)

### 5.13 IMMOBILIZATION OF TRANSFORMED BACTERIAL ISOLATES FOR HEAVY METAL UPTAKE

The transformed bacterial isolates combined with immobilization technique effectively remove the heavy metals. Microbial biomass consists of small particles with low density, poor mechanical strength and little rigidity. They are hard enough to withstand the application pressures, water retension capacity, porous to metal ion sorbate species and have high and fast sorption uptake even after repeated regeneration cycles reported by Alluri (2000). The cells are immobilized using calcium alginate gel. When free cells are used in a biosorption process, the separation stage represents a costly and technically difficult process. The problem of cell separation is removed when cells are immobilized. Due to immobilization, the biosorbent will have better shelf life and offers easy and convenient usage compared to free biomass which is easily biodegradable (Holan and Volesky 1994).

A great deal of heterogeneity exists among different bacterial species in relation to their number of surface binding strength for different ions and the binding mechanisms. (Paknikar *et al*., 2003). In the present work, during the immobilization process 78% of Chromium and 75% of nickel were removed by *Bacillus subtilis*. Whereas *Pseudomonas fluroscense* remove 90.7% of chromium and 87.2% of nickel. The percentage of heavy metal removal was greater during immobilization than the dried biomass. This is because, the dried biomass consists of small particles with low density, poor mechanical strength and little rigidity as reported by Leusch (2000). In the study of Johncy Rani *et al*., (2010), the adsorption capacity of the immobilized microbial isolates was greater than that of the dried biomass in *Bacillus*, *Pseudomonas* and *Micrococcus* respectively. In mixed culture the chromium is removed by 93%
and nickel 86%. The uptake of both chromium and nickel increased when both the transformed isolates are inoculated to the media. These were supported by Ashwani et al., (2008). They stated that microbial consortia are effective for degradation processes.

In the present study on immobilization with mixed culture proves that, the percentage of heavy metal removal increases when the culture was immobilized. To increase the further uptake, the mixed cultures can be used. This results correlates with the work of Ajao et al., (2011) where, the removal efficiency of physico-chemical parameters suggested the adoption of immobilized mixed culture of *Pseudomonas aeruginosa* and *Bacillus subtilis* for bioremediation of industrial effluents.

Thus in this work on bioremediation of textile effluent showed that, among the bacterial isolates *Pseudomonas flurosense* is efficient and in the fungal treatment, the *Aspergillus niger* is proved to be an efficient one. Compartively among the bacterial and fungal forms, the bacterial isolate *Pseudomonas flurosense* proved to be the best in reducing the physico-chemical parameters. In the transformed bacterial isolate treatment, the *Pseudomonas flurosense* was proved to be an efficient one than the *Bacillus subtilis*. Regarding the colour removal, the neem saw dust was most efficient and it removed 92% of colour. Studies on heavy metal resistance proves that *Pseudomonas flurosense* showed resistance for chromium and nickel up to 750 ppm. The immobilization studies proves that that, 91% of chromium and 81% of nickel was removed by *Pseudomonas flurosense*. In the mixed culture with *Bacillus subtilis* it removes 93% of chromium and 86% of nickel. Thus the transformed and mixed cultures of the *Pseudomonas flurosense* were proved to be the best in the treatment of textile effluent.

5.14 APPLICATION OF TREATED EFFLUENT TO CROP PLANTS.

Emerging trends of agricultural automation, and re-claimed and treated industrial effluent irrigation reduces stress through their ex-cressive micro element concentration (Adekola, 2002).
Rising trends of using the waste water (industrial effluents) for irrigation has the advantage of pollution removal where the pollutants are partly taken up by the plants and partly transformed in the soil without causing any damage. In many parts of the world, treated effluents have been successfully used for irrigation, and researchers have recognized its benefits (Adekola, 2003). Increased growth of crop plant irrigated with treated effluent is associated with the availability of increased organic matter, and both macro and micronutrients, especially total and available nitrogen in the treated effluent was reported by Kandiah, (1996). The industrial effluent as an alternative means of irrigation can offer a number of advantages. It contains various trace elements which can satisfy the need of micronutrients of crop plants. The Environment can be saved from its hazardous effects and utilizing the effluent the dependency on groundwater can be reduced to a great extent. Many scientists have reported the utilization of industrial effluent for the cultivation of variety of crops (Day, 1958, Juwarkar and Subramanyam, 1987).

5.14.1 Total Chlorophyll

The impact of untreated and treated effluent on chlorophyll is represented in the Table No: 19 . In the present study, the content of chlorophyll increases up to the day of 10, and then declines. This may be due to senescence of the leaf. Senescence is a coordinated deterioration of growth process that stimulates at full maturity and ultimately leads to death of the organisms. Decrease in chlorophyll content observed during senescence. In the senescence stage the production of chlorophyllase enzyme causes the degradation of chlorophyll. (Nayak et al., 1983). Also higher concentration of salts interfere with the denova synthesis of proteins the structural component of chloroplast (Sudhakar et al., 1991). Among the different treated effluent, the transformed bacterial treated effluent shows maximum chlorophyll content of 0.28 mg/L on Day 10. This coincides with the observations of Saha and Gupta (1998) and Diviate and Pandey (1981).
5.14.2 Protein

The impact of untreated and treated effluent on total protein is represented in the Table No: 20. In the present study, the content of protein increases with increase in days. The protein content was 44 mg/L at 20th day and was found to be maximum. After this the protein content decreases. The increase in protein content may be due to the synthesis of new proteins particularly stress proteins (Ohasi and Matsuoka, 1985, Christopher LaRosa et al., 1989). In the present study also the plants adjusts to the effluent stress by accumulation of proteins which corroborates with the work of Flowers and Yeo, (1983). The decrease in the protein content is due to the production of proteinase enzyme which causes the degradation of protein and convert it to amino acids. A characteristics decline in protein content after the senescence is due to the increase in the activity of protolytic enzyme (Huffaker 1990, James et al., 1993, Jones et al., 1994). Among the different treated effluent, the transformed bacterial treated effluent shows maximum protein content.

5.14.3 Total carbohydrate

The impact of untreated and treated effluent on total carbohydrate is represented in the Table No: 21. In the present study, the content of carbohydrate increases with increase in days. The carbohydrate content was found to be maximum at 20th day and the recorded value is 47 mg/L. After the 20th day, the carbohydrate content decreases. Similar reduction in carbohydrate content with increasing salt stress was also reported by Diefer Jeschke et al., (1986). The decreased carbohydrate at the harvest might be due to the translocation of available photosynthesis from leaves towards grain development and also may be because of senescence factor. Among the different treated effluent, the transformed bacterial treated effluent shows maximum carbohydrate content.

5.14.4 Amino acid

The impact of untreated and treated effluent on total amino acid is represented in the Table No: 22. In the present study, the amino acid content was gradually increases up to 20th day and
then declines. The trend of total free amino acid was inversely proportional to the trend of protein content indicating the incorporation of amino acid into protein synthesis. Neena Mehta and Bharathi (1983), reported similar trend in ground nut. The increased total free amino acid content at the later stage during the senescence may be due to the hydrolysis of protein by protolytic enzyme and this findings coincides with our work and also with the work of Nieman(1965) and Durga prasad et al., (1996). Among the different treated effluent, the transformed bacterial treated effluent shows maximum aminoacid content.

5.14.5 Proline

The impact of untreated and treated effluent on total proline is represented in the Table No: 23. In the present study, the content of proline increased up to 25th day and the recorded value is 0.320 mg/L. This result coincides with the work of Aspinall and Paleg (1981). Proline is reported to be an indicator of stress and it is synthesized during the stress condition. Hence the proline accumulation is the indicator of the stress reported by Green way and Setter, (1979), Hanson and Hitz, (1982) and Singh et al., (1987). Different stress factors may cause the activation of different protective mechanisms in plants. During the stress condition the synthesis of proline helps as a protector of cellular membrane and enzyme and it may also serve as a reservoir of energy and amino groups. Among the different treated effluent, the transformed bacterial treated effluent showed maximum proline content.

5.14.6 Total sugar

The impact of untreated and treated effluent on total sugar content of the plant is represented in the Table No: 24. In the present study, the content of total sugar increases with increase in days. After the period of day 20, the sugar content decreases and this may be decreased sugar content might be due to the translocation of available photosynthates from leaves towards grain development and also may be because of senescence factor.(Ohashi and Ohshima 1992). The geno types (Maize) with high
sugar content performed better which may be because of better maintenance of leaf water status through osmoregulation (Chetti et al., 1996). High content of total sugar maintains the assimilatory surface area and reduces the rate of leaf senescence (Hsiao et al., 1984). High amount of total sugar content was found in transformed bacterial effluent treatment.

5.14.7 Peroxidase

The impact of untreated and treated effluent on peroxidase activity is represented in the Table No: 25. In the present study, the enzymatic activity of peroxidase increases with increase in days up to certain time period of 20 days. At 20 days the peroxidase activity is 0.281 mg/g and is found to be maximum. After that the enzyme activity decreases. Peroxidase play the most essential role in scavenging ROS and protecting cells in crop plants. It decomposes Indole-3 acetic acid (IAA) and has a role in the biosynthesis of lignin and defense against biotic stresses by consuming hydrogen peroxidase. Sharma and Dubey (2000) found that mild drought stressed plants had higher chlorophyll activity than control grown plants but the activity declined at the higher level of drought stress. The transformed bacterial effluent treatment shows higher activity of peroxidases.

5.14.8 Superoxide dismutase

The impact of untreated and treated effluent on peroxidase activity is represented in the Table No: 26. In the present study, the enzymatic activity of superoxide dismutase increases up to 20 days of growth period. At 20 days the superoxide dismutase activity is 0.39 mg/g and is found to be maximum. After that the enzyme activity decreases. The over expression of superoxide dismutase scavenging enzyme resulted in abiotic stress tolerance in various crop plants due to efficient scavenging capacity. Also these enzymes used to obtain abiotic stress tolerance plants. (Mollar., 2001). The transformed bacterial effluent treatment shows higher activity of superoxide dismutase.
5.14.9 Reductase

The impact of untreated and treated effluent on reductase activity is represented in the Table No: 27. In the present study, the enzymatic activity of reductase increases with increase in days up to 20 days. At 20 days the reductase activity is 0.39 mg/g and is found to be maximum. After that the enzyme activity decreases. It is a potential enzyme and plays an essential role in defense system against ROS by sustaining the reduced status of GSH. It plays a crucial role in determining the tolerance of a plant under various stresses (Pan and Wu 2006). The increased reductase activity is found in leaf tissue of *C.arieitunum* (Eyidogan 2005). The transformed bacterial effluent treatment shows higher activity of reductase.