

## 2.0 REVIEW OF LITERATURE

The literature pertaining to the present study entitled “Bioremediation of methyl orange from aqueous solutions using *Oedogonium subplagiostomum* AP1” on decolourisation and degradation of textile dyeing effluent using algal biomass was presented under the following headings.

2.1 Textile dyes – An Overview

2.2 Textile dyeing pollution - Posing menace to life

2.3 Treatment of textile dyeing effluent

2.3.1 Bioremediation of dyes

2.3.1.1 Ideal characters for a microbe in bioremediation

2.3.1.2 Environmental factors affecting dye decolourisation

2.3.1.3 Algae as a biosorbent in the removal of dyes

2.4 Bioassay studies using treated wastewater

### 2.1 TEXTILE DYES – AN OVERVIEW

A dye is a coloured substance with chemical compounds which when applied to the fabric affix permanent colour and is not removed when washed with water or detergent or on exposure to sunlight (Reachakornkiji, 2004). A dye has two groups namely the chromophores which imparts colour to the compound and an auxochrome which serves to deepen or intensify the colour even though they impart no colour in the absence of chromophore. An auxochromome renders the molecule water soluble and enhance the affinity towards fibres. A chromogen (compounds with chromophore) without an auxochrome can never act as a dye (Gurses *et al.*, 2016).

Commercially, more than 1000 dyes are available with unique chemistry, structure and bonding mechanism. Many of the dyes can react chemically with the substrate forming strong bonds whereas the others are held by physical forces (Nigam *et al.*, 2000).

Dyes exhibit structural diversity and are classified in several ways. They are classified both by their chemical structure and application to the fiber type. Based on

the method of application on to fabric, the dyes are classified into acid, basic, direct, disperse, mordant, reactive, solvent and sulfur dyes. All these dyes have a common property to absorb light in visible region (Demirbas, 2009 and Adegoke and Bello, 2015).

**Acid dyes:** They are highly water soluble anionic dyes with one or more sulfonic acid groups/substituents and other acidic groups. The existence of sulfonic acid groups increase their solubility in water and gives negative charge to the dye molecule. They have better light fastness when compared to basic dyes. These dyes are applied to fibres such as silk, wool, nylon and they are not substantive to cellulosic fibres. Most of the synthetic food colours fall in this category.

**Basic dyes:** They are water soluble dyes which possess cationic functional groups such as  $-NR^{3+}$  or  $=NR^{2+}$ . They are generally amino and substituted amino compounds which are soluble in acid. They become attached to the fibers by formation of ionic bonds with fibers anionic groups. They are used to colour wool, silk, linen, hemp etc., without the application of a mordant. They give brilliant colour with exceptional fastness to acrylic fibres.

**Direct dyes:** They are water soluble with azo linkage ( $-N=N-$ ) and possess high molecular weight. They can be directly applied to the fabrics from an aqueous solution. They form hydrogen bonds with the fabrics during dyeing and have better fastness to light and washing. These dyes do not require any kind of fixing and hence the name direct dyes. They are used to colour cotton, linen, rayon, wool, silk and nylon.

**Mordant dyes:** These dyes do not dye the fabric directly but requires a fixing or binding agent known as mordant. They have the ability to form insoluble coloured complexes with metal ions. They are used for dyeing cotton, wool or other protein fibre.

**Reactive dyes:** These dyes will form a covalent bond when they react with the cellulosic fiber. They combine directly with the hydroxyl or amino group of the fibre. The dyed fabrics have high wash fastness properties. Once, they get attached with the fiber, it is difficult to remove them. Initially, these dyes were designed for cellulose fibers but some fiber-reactive dyes for protein and polyamide fibers are also available.

**Disperse dyes:** These dyes are relatively insoluble in water. Their structure is small, planner and non-ionic with attached polar functional groups, such as  $-\text{NO}_2$  and  $-\text{CN}$ . They are mainly used for the dyeing of polyesters, nylon and secondary cellulose acetate fibres. These dyes are ground into relatively fine powder in the presence of dispersing agents and used for dyeing.

**Solvent dyes:** These dyes are insoluble in water but soluble in alcohols, chlorinated hydrocarbons and liquid ammonia. They are mainly used for colouring plastics, synthetic fibers, gasoline, oils and waxes.

**Sulfur dyes:** They are applied to cotton from an alkaline reducing bath with sodium sulfide as the reducing agent. They possess sulfur-containing heterocyclic rings in their structure. These dyes are water insoluble but they are soluble in their reduced form and exhibit affinity to cellulose. They are low cost and have good fastness to light, washings and acids.

Based on their chemical structure they are classified into azo, anthraquinone, indigoid, phthalocyanines, di- and tri-aryl carbonium and nitro dyes (Gordon and Gregory, 1987 and Waring and Hallas, 1990).

**Azo dyes:** Azo dye makes them the largest group of synthetic colourants used in textile industries. They contain a minimum of one azo group ( $-\text{N}=\text{N}-$ ) attached to aromatic rings. These dyes possess bright red shades and often used in dyeing and printing industries. Azo dyes are complex aromatic compounds with significant structural diversity and are of great environmental concern because the reductive cleavage of azo linkage is responsible for the formation of amines, which are classified as toxic and carcinogenic.

**Anthraquinone dyes:** These dyes occupy the second most important class after azo dyes. They are also one of the oldest types of dye since they have been found in covering the mummies dating back to 4000 years. It is a polycyclic aromatic hydrocarbon derived from phthalic anhydride. In contrast to the azo dyes, which have no natural counterparts, all the natural red dyes were anthraquinones. It is used in textile and pulp industries and as a bird repellent. The lower tinctorial strength and reduced flexibility increase the production cost of anthraquinone dyes and hence, they are not as widely used as azo dyes.

**Indigoid dyes:** Indigoid dyes are an organic compound which contains carbonyl groups. They are vat dyes closely resembling indigo structure and used to dye cellulose and protein fibres as well as for cotton printing. Their resistance to light and water are high.

**Phthalocyanines:** Phthalocyanines are one of the novel chromophores which are structurally similar to the natural porphyrin pigments haemin and chlorophyll. These natural pigments have poor stability, but phthalocyanines have exceptional stability and tinctorially stronger. All the phthalocyanine compounds are blue to green in colour because substituents in their fused benzene rings exert only a minor sway on the color of phthalocyanines. They are widely used in printing inks and paints.

**Di- and tri-aryl carbonium dyes:** Di- and tri-aryl carbonium dyes and their heterocyclic derivatives comprise the oldest class of synthetic dyes. Diphenylmethane and Triphenylmethane dyes are the most important aryl carbonium dyes with exceptional brightness, high tinctorial strength and low light fastness.

**Nitro dyes:** Nitro dyes with simple chemical structure are of minor commercial importance and constitute only a fraction of the total dyes market. They are polynitro derivatives of phenols containing atleast one nitro group (ortho/para to the hydroxyl group). Initially, the nitro dyes were acid dyes which were used for dyeing the natural animal fibers i.e. wool and silk. Picric acid was the first synthetic nitro dye which imparted a greenish yellow colour to silk but could not be used for longer period of time because of its toxicity and poor fastness properties.

## **2.2 TEXTILE DYEING POLLUTION - POSING MENACE TO LIFE**

Water is a critical natural resource available in abundance on the earth for the survival of living organisms. Due to rapid industrialization and urbanization boundless amount of waste are discharged into aquatic and terrestrial ecosystem which leads to environmental pollution. In developing countries, environmental pollution is causing a serious problem, due to the discharge of various contaminants into land, ground water, streams, rivers and oceans and also improper management of vast amount of anthropogenic activities (Ghoreishi and Haghighi, 2003).

In the modern world, to produce quality and colourful products, especially azo dye stuffs have been produced widely and used in paper, textile, food, cosmetics and pharmaceutical industries. Among them, textile industries contribute as one of the largest consumer of dyes and pigments and accounts for 80% of total production. Indian textile industry is the world's second largest after China with about 1200 medium to large scale textile mills and 3000 garment manufacturing units employing about 3 million people. India exports to 162 countries which accounts to 38% of Indian economy. The textile industry utilizes voluminous amount of water and variety of chemicals during production process. For the process of dyeing, about 200L of water is used to dye 1kg of fabric and the waste water produced during this process impart strong colour (Namasivayam and Yamuna, 1992).

Textile dyes are synthetic, aromatic organic compounds which have extensive application as colourants in dyeing and printing industries. More than 1000 dyes were commercially available worldwide with an annual production of over  $7 \times 10^5$  metric tonnes and 5-10% of the dye is lost in the industrial effluent. Among the dyes available, azo dyes seem to be non-biodegradable, persistent and recalcitrant. When the dye based waste water constituting a complex mixture of polluting agent is released into an environment, they pose several health hazards to the flora, fauna and also to the ecosystem which has become a critical environmental concern (Smaranda *et al.*, 2011 and Abdulah *et al.*, 2005).

When these coloured effluent possessing dyestuffs are released into aquatic bodies they affect their aesthetics, obstruct light penetration and oxygen transfer which reduces the photosynthetic activity of algae, its primary productivity and also stop the reoxygenation capacity of the receiving water. The dissolved oxygen concentration and BOD level of the water bodies are adversely affected and also cause acute toxicity to the aquatic flora and fauna (Khasri and Ahmad, 2018).

The untreated dyeing effluents that are straightly used in agriculture have a serious impact on environment and human health (Pourbabee *et al.*, 2006). The textile dyes leads to bioaccumulation that may incorporate into food chain and affect human health. Azo dye exhibits the activity of tyrosinase enzymes that leads to inhibition of melanin and results in hypo pigmentation (Dubey *et al.*, 2007).

If the textile effluents can seep into the aquifer and pollute the ground water the metals in the effluents may increase fertility of the sediment, water column and consequently leads to eutrophication, which in open water can progressively lead to oxygen deficiency, algal bloom and death of aquatic life (Purdom and Anderson, 1980).

Polluted aquatic body is being the source of acute or chronic toxicity for aquatic organisms, mainly fish. The formation of micronuclei has been observed in fish erythrocytes, as a consequence of exposure to environmental and chemical contaminants of cytotoxic, genotoxic, mutagenic or carcinogenic action (Souza and Rodriguez, 2007). The formation of morphological nuclear alterations, nuclear buds, fragmented apoptotic and bi-nucleated cells has been identified in the erythrocytes of fish which seems to be the possible indicators of genotoxicity. Fish gills, vital part of the body are multifunctional and are the site of direct uptake of toxicants as they constantly exposed to the environment. Histopathological changes in gills directly affect the haematological parameters as well as kidney functioning (Vigliano *et al.*, 2006).

Dyes undergo alteration in their chemical structure and results in the formation of new xenobiotic compounds, which may be more or less toxic than the parental compounds (Vijaya and Sandhya, 2003). They can also cause human health disorders such as nausea, hemorrhage, ulceration of the skin and mucous membrane and also severe damage to kidney, reproductive system, liver, brain and central nervous system. Even some dye causes allergic dermatitis, respiratory diseases, eye and skin irritation, cancer and mutation in man (Kadirvelu *et al.*, 2002).

Dyes cause severe health defects such as splenic sarcomas, hepatocarcinoma and bladder cancer with nuclear anomalies in animal models and chromosomal aberrations in cultured mammalian tissues. The long term exposure of workers to dyes may cause health issues such as triple primary cancers, involving kidney, urinary bladder and liver (Verma *et al.*, 2003).

Hence, government, global institutions and local bodies should take utmost care to utilize the advance resources to balance the environment for living and also to initiate the breathed intellectuals to live eco-friendly.

## **2.3 TREATMENT OF TEXTILE DYEING EFFLUENT**

The decolourization of textile waste water is still a major environmental concern because of synthetic dyes, which are difficult to be removed by conventional treatment systems (Robinson *et al.*, 2001).

Traditional methods for the cleanup of pollutants usually involve the removal of unwanted materials through filtration, sedimentation, floatation, irradiation, ozonation, anion exchange resins, flocculation, neutralization, activated carbon etc. Additionally the physical and chemical remediation techniques requires more energy and chemicals and also need disposal site or extra treatments for concentrated pollution as well as they are often time consuming and expensive (John, 2006).

Biological remediation is considered more efficient in term of its long lasting benefits and having almost no harmful effects on environment. In addition, it would be cheaper and eco-friendly than other different physicochemical techniques tested (Saratale *et al.*, 2001).

### **2.3.1 Bioremediation of dyes**

Successful bioremediation requires not only the knowledge of the microorganisms that can degrade a particular compound, but also an understanding of the pathways involved in the degradation both at physiological and molecular levels. Thus bioremediation uses living systems especially microbes that constitute the most extraordinary reservoir of life in the biosphere to reclaim the waste at the site (Dawkar, 2008).

#### **2.3.1.1 Ideal characters of microbe in bioremediation**

Genetic, biochemical and ecological abilities of microbes play a significant role in remediation. Faster kinetic rate is economical and biomass with slow specific growth rate responds more favourably to shock loadings. Appropriate reactor designs can control microbial system under various operating conditions to give wastewater of acceptable quality. Microbial degradation depends on several conditions namely

- ▶ Presence of microbes with the capacity to degrade the target components
- ▶ Substrate being accessible to the microbes and capable of being used as energy and carbon source.

- ▶ Presence of inducer to cause synthesis of specific enzymes for the target compounds.
- ▶ Presence of an appropriate electron acceptor-donor system.
- ▶ Ideal moisture and pH for microbial growth.
- ▶ Nutrients for microbial growth and enzyme production.
- ▶ Optimum temperature to support microbial activity.
- ▶ Absence of toxic substance.
- ▶ Microbes to degrade metabolic products.
- ▶ Microbes to prevent transit of toxic intermediate products.
- ▶ Ideal conditions to minimize competitive organisms for those conducting desired reactions

Thus the richness and evenness of the microbial diversity in an ecosystem reflects the capability to degrade or transform the contaminants (Rajaendran and Gunasekaran, 2007).

### **2.3.1.2 Environmental factors affecting dye decolourisation**

Culture conditions affect the physiology, expression and activity of the microbes which is essential for effective dye degradation. Decolourisation ability of the fungi can be substantially increased by optimizing the operational conditions such as nutrient content of the culture media, age of the fungus, environmental conditions, pH, temperature, contact time, cell age etc. (Razi *et al.*, 2017).

#### **pH**

pH is one of the very important factor in most biological processes. Further, the pH dependence on degradation is highly related to functional groups present in the biomass and also on the other reactive moieties present in the solution. For biosorption of textile dye pH is one of the most important environmental factor which influences not only the dissociation site of the biomass surface but also the solution chemistry of the textile dye. The biosorption capacity of the dye increases with the increasing pH of the sorption system but not in a linear relationship. On the other hand, higher pH can cause precipitation which will leads to decreased biosorption efficiency of the dye (Etim *et al.*, 2012, Brahim *et al.*, 2014 and Bharathi and Ramesh, 2013).

## **Temperature**

Temperature also has an influence on the biosorption of textile dye. Under a certain range of temperature ion exchange mechanism also exists. Biosorption process is usually not operated at high temperature because it will increase the operational cost. Temperature is well known to play an important role in both biosorption process and equilibrium uptake of dyes by microorganism. For the treatment of textile effluent temperature should be optimized and maintained. Temperature above 40° C may yield colloidal solution which could not be cleared either by filtration or centrifugation (Chellababu *et al.*, 2008).

## **Contact Time**

Contact time is an important parameter in all transfer phenomena including adsorption. Consequently, it is important to study its effect on the capacity or removal of textile dye. The removal efficiency of the dye is increased with increase in contact time. Hence, it is necessary to optimize the contact time, considering the efficiency of desorption and regeneration of the biomass (Salleh *et al.*, 2012).

## **Initial dye concentration**

The uptake of the textile dye increases along with the increase in initial concentration when the amount of biomass is kept unchanged. Contrary to that, biosorptive capacity of the textile dye is inversely proportional to the initial concentration of the biomass when it is kept constant. As the dye concentration is increased in the culture medium a decline in colour removal was attained (Khalaf, 2008).

## **Biosorbent concentration**

Biosorbent concentration is one of the important factors in order to determine the adsorbent quantity for a given amount of adsorbate at the operating conditions. The increase in dye removal along with the increase in concentration of adsorbent dose favours the deposition of dyes on the surface of the adsorbent that will result in the increase of dye removal percentage (Adeyemo *et al.*, 2015).

## Sources of Energy

### Carbon Source

The carbon source commonly includes glucose, fructose, xylose, sucrose, maltose, mannitol, starch and galactose. Glucose is the most widely used carbon source which acts as an important source of energy. For the biosorption of living cells the addition of glucose and other trace elements into a biosorption system will enhance the growth of living cells and facilitate dye biosorption (Kalaiarasi and Lavanya, 2010).

### Nitrogen Source

Nitrogen source commonly includes urea, ammonium chloride, potassium nitrate, sodium chloride etc. Different nitrogen source influences the rate of decolourisation of the textile dye. There are few literatures which proved that nitrogen source also inhibit the decolourising efficiency. A strong inhibitory effect was recorded at higher levels for all additional nitrogen sources under investigation (Zhang *et al.*, 1999).

#### 2.3.1.3 Algae as a biosorbent in the removal of dyes

Algae are photosynthetic organisms distributed in different habitats and in all parts of the world. Algae have an ability to use the pollutants as nutritional source when they are at high level in the natural environment and degrade them enzymatically. Also when light is provided algae do not require oxygen for their photosynthetic activity and hence fixes carbon dioxide in water system. Filamentous cyanobacteria or diazotrophs can fix atmospheric nitrogen and fewer algae or photoautotrophs which do not require carbon source, utilize atmospheric air thereby proving its efficiency in treatment process (Silva-Stenico *et al.*, 2012).

Algal species are commonly recognised as an important source for reducing pollutants from the wastewater because it can adapt to different environmental conditions like pH, temperature and pressure even at extreme variations because of their wide biochemical versatility. Algae are capable of developing tolerance to contaminants that induce a toxic effect on their vital functions. Algae have the

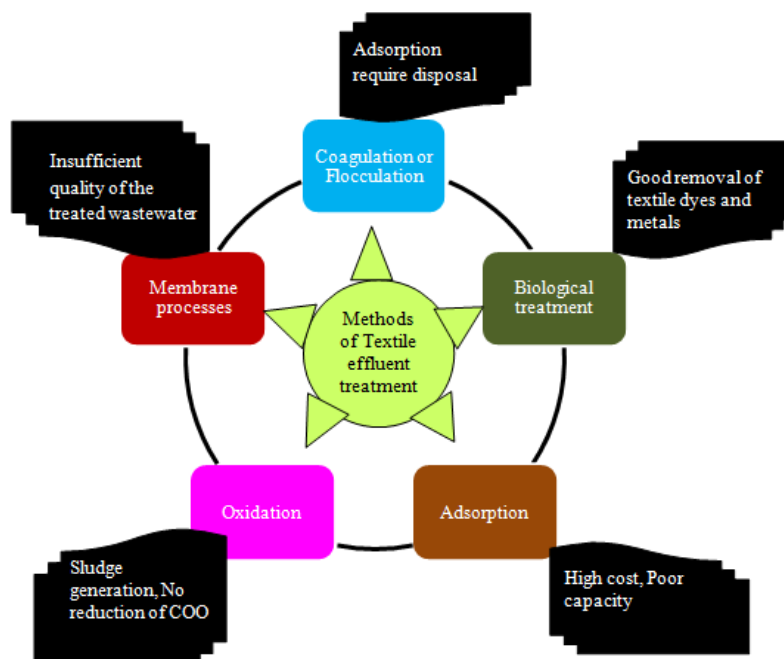
genetic information that are capable of producing enzymes involved in transformation of contaminants that tolerate extreme conditions. They consume different nutrients, which can also be pollutants for natural environments when their concentration is at a higher level. They do not cause desirable smell as much as bacteria. Therefore, using algae for bioremediation offers a number of advantages (Omar *et al.*, 2018).

Moreover, algae proves as an ideal candidate in bioremediation, since it is an promising source of biomass with high protein supplement, ability to utilize marine, fresh or waste water, non-requirement of fertile land, high tolerance to toxicants, ability to grow autotrophically or heterotrophically, large surface area/ volume ratio, phytochelatin expression and potential for genetic manipulation (Pansamriut, 2007).

Both viable and nonviable algae have been used as a biosorbent in textile waste water remediation and also offer a solution to global environmental problems such as greenhouse effect. The dyes in water bodies cause severe toxic effect to the aquatic life forms but it does not affect the algal forms which can proliferate in eutrophicated conditions. The algae possess polyphosphate bodies which enable to store the toxicants in the storage pool and also help in detoxification mechanism (Choudhary and Pradhan, 2015).

The use of microalgae is desirable in wastewater treatment, since they generate biomass for biofuel production with concomitant carbondioxide sequestration, there no secondary pollution as long as the biomass produced is reused and efficient recycling of nutrients and also scavenges the dyes (Abd-El-Kareema and Tahab, 2012).

Thus bioremediation by algae provides a cheap and more eco-friendly approach for removal of the contaminants/pollutants in the aquatic and terrestrial environment when compared to physicochemical techniques. For any kind of treatment process, the algae or their enzymatic activities can be considered as the most promising microorganism for bioremediation processes (Adams *et al.*, 2015). Figure - 1 displays the methods of treatment of textile effluent.



**Figure 1 - Methods of textile effluent treatment**

Diverse algal species have been investigated for biodegradation of textile dye stuffs due to their excellence in large biomass production, hostile growth and high surface to the cell ratio. Algae have been extensively studied to degrade textile dye due to their extracellular oxidoreductive, non-specific and non-stereo selective enzyme system (Azza *et al.*, 2013).

Allouche and Yassaa (2018) evaluated the adsorption potential of methylene blue from aqueous solution using *Posidonia oceanica* at optimum conditions. A maximum percentage removal of colour was obtained at pH 5 at 20°C. Equilibrium isotherm data were analysed using the Langmuir and Freundlich isotherms and the adsorption equilibrium of methylene blue suited well with Langmuir model. The sorption data was well described by the pseudo-second order model.

Mokhtar *et al.* (2017) investigated the potential of *Euchema spinosum* in the removal of methylene blue from aqueous solution. The alga was examined for the effect of various operational parameters such as pH (2-11), biosorbent dosage (0.2-1.2g/L) and initial dye concentration (50-200mg/L). Maximum removal of MB (84%) was achieved at pH 8 with 1.4g/L adsorbent dose within 1h of incubation period. Experimental data were compiled well with Langmuir and Freundlich isotherm and the kinetic data best fitted to the pseudo-second order kinetic model.

Ebrahim *et al.* (2016) aimed to evaluate the efficiency of *Sargassum glaucescens* in the adsorption of reactive orange 16 at various optimization parameters such as pH, initial dye concentration, adsorbent dose and contact time. The maximum decolourisation was achieved at 25mg/L dye concentration, 10mg/L biosorbent dose at pH 5 within 120 minutes. The equilibrium constant was more consistent with Freundlich isotherm proving *S. glaucescens* as an economic and effective absorbent in treatment of dyes.

Vijayaraghavan *et al.* (2015) examined the potential of *Gracilaria corticata* in the removal of crystal violet from aqueous solution with respect to pH, biosorbent dosage and initial dye concentration. The maximum biosorption was recorded at pH 8 with 5 g/L biosorbent dosage and 1000mg/L which is the initial dye concentration. The equilibrium isotherms were described using the Langmuir, Freundlich, Redlich-Peterson, Toth and Sips adsorption models. Langmuir model well fits with the equilibrium data. Fourier-transform infrared spectroscopy confirmed the dye biosorption mechanism as electrostatic interaction between the negatively charged seaweed surface and positively charged crystal violet. Hence, *G. corticata* seems to be an effective adsorbent for the removal of crystal violet from aqueous solution.

Sharma *et al.* (2014) studied the effect of *Spirulina platensis*, *Chlorella vulgaris*, *Anacystis nidulans* and *Oscillatoria agardhii* to decolourize the textile dyes namely methyl red, methyl orange, crystal violet, erichrome black and malachite green respectively. *S. platensis* was found to have more decolourisation activity (77%) at pH 5 for methyl red than other algal species.

Khataee *et al.* (2013) investigated the removal of acid orange 7, basic red 46 and basic blue 3 from contaminated water by *Spirogyra* species at different physicochemical parameters such as contact time (20 - 140min), pH (2 - 12), initial dye concentration (5 - 45mg/L), biosorbent dose (1 - 6g/L) and temperature (288-318K) respectively. The maximum dye uptake occurred within 1h of incubation time with 1g/L of biosorbent dose amended in 15mg/L dye concentration at 25°C. The biosorption of the dyes onto the dried *Spirogyra* biomass showed spontaneous and endothermic process. The pseudo-second order kinetic model best fits with the

experimental kinetic biosorption data and the adsorption process followed the Freundlich isotherm model.

Kousha *et al.* (2012) studied the application of algal species in the removal of acid black from wastewater at different biomass dosage ( $0.5 - 1.5 \text{ mg L}^{-1}$ ), dye concentration ( $10 - 50 \text{ mg L}^{-1}$ ), pH (2-6) and incubation time (20 - 80min). Under optimum conditions, the maximum removal efficiencies of acid black was achieved for *Cystoseira indica* and *Gracilaria persica* respectively. Presence of functional groups such as hydroxyl, carboxyl, amino and phosphate found on the surface of which algal cell surface are considered to be responsible for separation of contaminants from water (Srinivasan and Viraraghavan, 2010).

Mona *et al.* (2011) studied the decolourisation of tri-phenylmethane and crystal violet onto waste biomass of *Nostoc linckia*. The batch mode experiment was performed to determine the kinetic behaviour of the dye in aqueous solution allowing the computation of kinetic parameters. Influence of optimization parameters such as temperature ( $25 - 35^\circ\text{C}$ ), pH (4 - 8), initial dye concentration ( $10 - 200 \text{ mg/L}$ ) and biosorbent dose ( $0.2 - 0.4 \text{ g}$ ) on dye removal were examined. The ability of the biomass to decolourise the dye was maximum at pH 8, temperature  $35^\circ\text{C}$ ,  $200 \text{ mg/L}$  initial dye concentration and  $0.2 \text{ g}$  biosorbent dose. Adsorption of the dye on cell surface was further confirmed by scanning electron micrographs of the biomass before and after dye loading. FT-IR studies revealed that decolourisation was mediated mainly by functional groups like hydroxyl, amide, carboxylate, methyl and methylene groups present on the cell surface.

Rajeshkannan *et al.* (2010) studied the optimization parameters such as temperature, adsorbent dose, contact time, adsorbent size and agitation speed for acid blue 9 removal from aqueous medium using *Turbinaria conoides*. The optimum conditions for maximum removal of acid blue 9 from an aqueous solution of  $100 \text{ mg/l}$  were found as follows: temperature ( $33^\circ\text{C}$ ), adsorbent dose ( $3 \text{ g/l}$ ), contact time (225 min), adsorbent size ( $0.177 \text{ mm}$ ) and agitation speed (226 rpm). The pseudo-second order model well suits with the system and from equilibrium studies, the Freundlich and Redlich-Peterson isotherm fits the data well.

## 2.4 BIOASSAY STUDIES USING TREATED WASTEWATER

Due to rise in human population and their increasing demands for modernization, made the ecosystem polluted. Industrialization ended in the disposal of untreated wastewater on land which has direct impact on soil fertility and agricultural productivity. There has been a strong global awakening during the last few decades regarding the proper management of existing natural resources. Treatment of wastewater using biological methods proves to be eco-friendly but, there is need to assess the toxicity of end products formed after the treatment process. The toxicity of azo dyes and their metabolic intermediates before and after microbial treatment has been reviewed by many researchers using algae, protozoa, invertebrates, bacteria, yeast, fungi and agricultural products. They highlighted in their reports that textile dyes and wastewater have toxic consequences on the germination rates and biomass of various plant species, affects the metabolic activities of fauna and microflora of the ecosystem. Therefore, treatment of industrial wastewater containing azo dyes is essential prior to their final discharge into aquatic bodies. The treated wastewater can be used for multipurpose operations globally (Pokharia and Ahluwalia, 2015).

Asses *et al.* (2018) performed the phytotoxicity and microbial toxicity assays to assess the toxicity of congo red before and after degradation by *Aspergillus niger* using *Zea mais* and *Solanum lycosicum* seeds and *Bacillus cereus* ATCC 11 and *Escherichia coli* ATCC 10536 respectively. Compared to water treatment, congo red before degradation reduced significantly the germination rate, shoot and root length of both plants and the metabolites generated after the congo red degradation are less toxic than the crude dye. Inhibition zone was observed with untreated congo red dye solution and no zone of inhibition was noticed in dye degraded metabolites.

Tripathy and Rao (2015) studied the mitotic aberrations induced by orange red (a food additive dye) on root tip cells of onion (*Allium cepa*). Gradual decondensation of chromosomal arms, abnormal metaphase and anaphase, unequal cytokinesis and karyokinesis and formation of bi-nucleated cells were observed.

Du *et al.* (2015) carried out the phytotoxicity test to evaluate the effect of methyl orange and its degradation products on the seeds of Chinese cabbage (*Brassica pekinensis*). The root length, shoot length and the germination percentage were recorded in the seedlings. Results revealed that the methyl orange treated with *Aeromonas* revealed better growth whereas the seedlings grown with methyl orange solution were withered and became yellow after one week and dead within two weeks indicating its toxicity.

Saha *et al.* (2014) assessed the reuse of treated dye wastewater on the selected crops such as Indian spinach, steam amaranth, pea, wheat and tomato. They also studied the effect of growth parameters, nutritional contents and yield attributes of the selected plants. The result suggests that the application of treated dyeing wastewater is beneficial for irrigation and can be the sustainable solution to industrial pollution.

Dhanve *et al.* (2014) studied the cytotoxic and genotoxic effect of the untreated and treated textile effluent on *Allium cepa*. Exposure of root tip cells of *A. cepa* to untreated effluent exerted occurrence of different chromosomal aberrations, diminished root growth, inhibition of root elongation, suppression of mitotic activity and stunted root length. The biodegradation product did not show any inhibitory effect on root growth and elongation indeed the roots grew well in the presence of degradation product.

Tripathy and Patel (2014) investigated the mitotic abnormalities in root meristem cells of *Allium cepa* induced by a fabric reactive dye, turquoise blue (Procion MX). Inhibition of root growth, root depression and abnormal mitotic cells were observed indicating the genotoxic assault of the dye on chromosomal condensation mechanism resulting in very unusual long arms in rapidly dividing meristematic cells.

Dhanve *et al.* (2014) studied the effect of the untreated and textile effluent with *Exiguobacterium* sp. RD3 on the behavioural changes, mortality rate and haematology of aquatic fish, *Acanthopsis choirorhyncus*. The fishes exposed to untreated effluent showed anxious movement, higher mortality rate and the haematology of the blood cells exhibited abnormalities in the shape of erythrocytes

and formation of micronuclei. In contrast, the biodegradation product of the effluent did not induce any observable hazardous health effect on the fish.

Amte and Mhaskar (2013) evaluated the haematological parameters such as Hb, RBCs, WBCs, PCV, MCH and MCHC in *Oreochromis ossambicus* exposed to various concentrations of textile dyeing effluents (both treated and untreated). The levels of the haematological parameters were altered in fishes exposed to untreated dye solution when compared to treated effluent.

Barot and Bahadur (2011) reported the histotoxic effects of acid orange 7, an azo dye on the tissues such as gill, kidney and liver in the fingerlings of *Labeo rohita*. In the gills hyperplasia, epithelial lifting, hooked secondary gill lamellae, haemorrhage in the primary gill lamellae and erosion of secondary gill lamellae were observed. The kidney showed shrunken glomeruli, increased periglomerular space, wide peritubular space, degenerated tubules, fat deposition and infiltration of blood cells in the lumen of the renal tubules. On the other hand, the liver showed disorganized hepatocytes, focal necrosis, haemorrhage, karyolysis and karyorrhexis.

Jadhav *et al.* (2010) carried out *Allium cepa* test for the *in situ* monitoring of cytogenotoxicity of the textile effluent before and after treatment. The biotreated cells exposed to various concentrations exhibits the mitotic index in the range of the control. The genotoxicity tests showed that the percentage of aberrant mitotic cells caused by different effluent concentrations of the untreated effluent was significantly different from that of the control.

Saratale *et al.* (2009) studied the decolourisation of scarlet R and its degraded products on the germination percentage, plumule and radical length of *Phaseolus mungo* and *Sorghum vulgare*. Phytotoxicity studies revealed no toxicity on the biodegraded products of scarlet R by Consortium-GR (*Proteus vulgaris* NCIM – 202 and *Microccus glutamicus* NCIM - 2168) suggesting the potential application of the treated waste water in agricultural purposes.

Kalyani *et al.* (2008) evaluated the effect of red BLI treated with *Pseudomonas* sp. SUK1 on the growth of *Sorghum vulgare* and *Phaseolus mungo*.

Phytotoxicity testing with the seeds of *S. vulgare* and *P. mungo* showed more sensitivity towards the red BLI while the products obtained after dye decolourisation does not have any inhibitory effects. Jadhav *et al.* (2008) reported the effect of direct red 5B and its degraded products using *Comamonas* sp. UVS on the growth of *Triticum aestivum* seeds. The germination of *T. aestivum* seeds was inhibited with direct red 5B treatment but not with the treatment of dye degradation products indicating its non-toxic nature.

Telke *et al.* (2008) studied the effect of reactive red 141 and its decolourised products using *Rhizobium radiobacter* MTCC 8161 on the growth of *Triticum aestivum* and *Oryza sativa*. Germination percentage of both the plants was less with reactive red 141 treatments as compared to their degradation products and distilled water. The reactive red 141 significantly reduced the length of plumule and radical than its degradation product which indicated the less toxicity of the degraded products.

The wastewater should be explored for the agricultural and aquacultural purposes after treating with biological resources which paves a platform for an eco-friendly approach to mankind.

A detailed description of the methodology adopted for the present study is given in the following chapter.