Chapter 4
Dyes—Environmental Impact and Remediation

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Contents

Abstract Dyes are an important class of synthetic organic compounds used in many industries, especially textiles. Consequently, they have become common industrial environmental pollutants during their synthesis and later during fibre dyeing. Textile industries are facing a challenge in the field of quality and productivity due to the globalization of the world market. As the highly competitive atmosphere and the ecological parameters become more stringent, the prime concern of the textile processors is to be aware of the quality of their products and also the environmental friendliness of the manufacturing processes. This in turn makes it essential for innovations and changes in these processes, and investigations of appropriate and environmentally friendly treatment technologies or their residues. The large-scale production and extensive application of synthetic dyes can cause considerable environmental pollution, making it a serious public concern. Legislation on the limits of

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colour discharge has become increasingly rigid. There is a considerable urgent need to develop treatment methods that are effective in eliminating dyes from their waste. Physicochemical and biological methods have been studied and applied, although each has its advantages and disadvantages, with the choice being based on the wastewater characteristics, available technology and economic factors. Some industrial-scale wastewater treatment systems are now available; however, these are neither fully effective for complete colour removal nor do they address water recycling.

This chapter outlines the background of dye chemistry, the application areas and the impact of dyeing effluents in the environment. The processes/techniques being implemented and developed for wastewaters remediation are revisited.

Keywords Dye • Textile industry • Decolourisation • Physico-chemical treatment • Bioremediation

4.1 Introduction

Environmental pollution is one of the major and most urgent problems of the modern world. Industries are the greatest polluters, with the textile industry generatings high liquid effluent pollutants due to the large quantities of water used in fabric processing. In this industry, wastewaters differing in composition are produced, from which coloured water released during the dyeing of fabrics may be the most problematic since even a trace of dye can remain highly visible. Other industries such as paper and pulp mills, dyestuff, distilleries, and tanneries are also producing highly coloured wastewaters. It is in the textile industry that the largest quantities of aqueous wastes and dye effluents are discharged from the dyeing process, with both strong persistent colour and a high biological oxygen demand (BOD), both of which are aesthetically and environmentally unacceptable (Wang et al. 2007). In general, the final textile waste effluent can be broadly categorized into 3 types, high, medium and low strength on the basis of their COD content (Table 4.1).

The textile industry plays a major role in the economy of Asian and other countries. In India, it accounts for the largest consumption of dyestuffs at ~80% (Mathur et al. 2003), taking in every type of dye and pigment produced, this amounts to close to 80 000 tonnes. India is the second largest exporter of dyestuffs, after China. Worldwide, ~10^6 tons of synthetic dyes are produced annually, of which $1–1.5 \times 10^5$ tons are released into the environment in wastewaters (Zollinger 1987). This release is because not all dye binds to the fabric during the dyeing processes; depending on the class of the dye, the losses in wastewaters can vary from 2% for basic dyes to as high as 50% for reactive dyes, leading to severe contamination of

Table 4.1 Some characteristics of typical textile effluents

Wastewater type	COD ($mg \cdot L^{-1}$)	Conductivity (μScm^{-1})
High strength	1500	2900
Medium strength	970	2500
Low strength	460	2100

surface and ground waters in the vicinity of dyeing industries (O'Neill et al. 1999). It is estimated that globally 280 000 tons of textile dyes are discharged in textile industrial effluent every year (Jin et al. 2007). Apart from the aesthetic point of view, dyes are undesirable because they can affect living creatures in the water discharged as effluent into the environment. Industrial effluents containing synthetic dyes reduce light penetration in rivers and thus affect the photosynthetic activities of aquatic flora, thereby severely affecting the food source of aquatic organisms. The thin layer of discharged dyes that can form over the surfaces of the receiving waters also decreases the amount of dissolved oxygen, thereby affecting the aquatic fauna. Furthermore, dye-containing effluents increase biochemical oxygen demand. Dyes are in general stable organic pollutants that persist in the environment, and concern has been raised that such artificial compounds are xenobiotic. Therefore, methods for their degradation have been increasingly explored and development. Despite the number of successful systems employing various physicochemical and biological processes, economical removal of colour from effluents remains a major problem. Since these concerns about the environment are gaining momentum, it is necessary to develop better economically and environmentally friendly treatment technologies. Among the current pollution control technologies, biodegradation of synthetic dyes by various microbes is emerging as an effective and promising approach. The bioremediation potential of microbes and their enzymes acting on synthetic dyes has been demonstrated, with others needing to be explored in the future as alternatives to conventional physicochemical approaches (Husain 2006; Ali 2010). It is obvious that each process has its own constraints in terms of cost, feasibility, practicability, reliability, stability, environmental impact, sludge production, operational difficulty, pre-treatment requirements, the extent of the organic removal and potential toxic by-products. Also, the use of a single process may not completely decolourise the wastewater and degrade the dye molecules. Even when some processes are reported to be successful in decolourising a particular wastewater, the same may not be applicable to other types of coloured wastewaters. Certainly, the effective removal of dye from industrial coloured wastewater is a challenge to the manufactures and researchers, as some of the processes are neither economical nor effective. The amount of water consumed in textile industries must also be considered because the traditional textile finishing industry consumes ~100 L of water in the processing of a kg of textile material. Consequently the potential of water re-use should be an objective when applying a particular wastewater treatment.

In this chapter, all these issues will come under focus and discussion, based on the theoretical and practical aspects of each of them.

4.2 Dye Structures and Properties

The textile dyeing industry has been in existence for over 4000 years. In ancient times, dyes were obtained from natural sources and not everyone could possess coloured fabrics. For example, during the early Roman Empire period, only kings

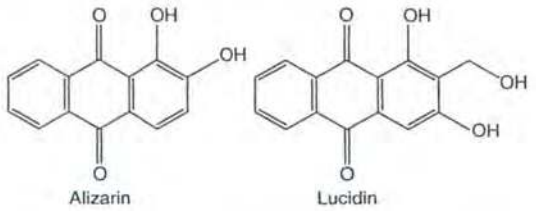

Fig. 4.1 Example of two natural extracts obtained from madder-rot: Alizarin and Lucidin

and priests could wear purple dyed fabrics while in the middle-ages, scarlet dyed fabrics were reserved exclusively for important members of the clergy. Natural colouring agents are mainly of inorganic origin (clays, earths, minerals, metal salts, and even semi-precious stones, such as malachite) or organic dyestuffs traditionally divided into 2 groups, one of animal and the other of plant origin (Ackacha et al. 2003). Undoubtedly, botanical sources were the most important, but a wide variety of other organisms was used, including lichens, insects and shellfish. Organic dyes present a broad spectrum of compounds with different physical and chemical properties. Among them, anthraquinone red colorants (e.g. cochineal, lac dye or madder root) are of special interest. Madder root has a long tradition as a dyestuff because of its bright red colour. The red pants of Napolean's army and the red coats of the English soldiers in the 18/19th century were dyed with madder. However, extracts of Madder root contain mainly alizarin (1,2-dihydroxy-anthraquinone) and several by-products, in which lucidin (1,3-dihydroxy-2-hydroxymethyl-anthraquione) is of the special concern because it has proved to have mutagenic character, severely constraining the use of Madder root (Fig. 4.1). Moreover, not every shade is directly available from a natural source. Synthetic dyes quickly replaced the traditional natural dyes. They cost less, offered a vast range of new colors, and imparted improved properties to the dyed materials.

In 1856, William Henry Perkin accidentally discovered the world's first commercially successful synthetic dye. By the end of the 19th century, 10 000 new synthetic dyes had been developed and manufactured. Nowadays, India, the former USSR, Eastern Europe, China, South Korea and Taiwan consume ~600 thousand tons (kt) of dyes per annum. Since 1995, China has been the leading producer of dyestuffs, exceeding 200 kt per annum (Wesenberg et al. 2003).

A large variety of dyestuffs is available, which can be natural or synthetic substances, but synthetic dyes are commonly used for textile fibres, whereas natural dyes tend to be reserved for the food industry.

A dye can generally be described as a coloured substance that has an affinity for the substrate to which it is being applied. It is a coloured because it absorbs in the visible range of the spectrum at a certain wavelength (Table 4.2). In general, a small amount of dye in aqueous solution can produce a vivid colour, which is related with the high molar extinction coefficients. Colour can be quantified by spectrophotometry (visible spectra), chromatography (usually high performance liquid, HPLC) and high performance capillary electrophoresis (Fig. 4.2).

Table 4.2 Colours of the visible spectrum: wavelengths and frequencies intervals

Colour	Wavelength interval (nm)	Frequency interval (THz)
Red	~700–635	~430–480
Orange	~635–590	~480–510
Yellow	~590–560	~510–540
Green	~560–490	~540–610
Blue	~490–450	~610–670
Violet	~450–400	~670–750

The major structure element responsible for light absorption in dye molecules is the **chromophore** group, i.e., a delocalized electron system with conjugated double or simple bonds (Gomes 2001). Chromophores frequently contain heteroatoms as N, O, and S, with non-bonding electrons. Common chromophores include –N=N– (azo), =C=O (carbonyl),=C=C=, C=NH, –CH=N–, NO or N–OH (nitroso), $–NO_2$ or NO–OH (nitro) and C=S (sulphur). As a complement to the electron acceptors action are the groups called **auxochromes**, which are electron donors generally on the opposite side of the molecule and their basic function is to increase colour. Indeed, the basic meaning of the word auxochrome is *colour enhancer.* Some auxochromes include $–NH_3$, –COOH, HSO_3 and –OH. These groups also have the important property of giving a higher affinity to the fibre. The chromogen, which is an aromatic structure (normally benzene, naphtalene or anthracene rings), is part of a chromogen-chromophore structure along with an auxochrome. Synthetic dyes exhibit considerable structural diversity and thus possess very different chemical and physical properties. The chemical classes of dyes more frequently employed on an industrial scale are azo, anthraquinone, indigoid, xanthene, arylmethane and phthalocyanine derivatives (Fig. 4.3). Nevertheless, it needs to be emphasized that the overwhelming majority of synthetic dyes in current use are **azo** derivatives. The colour range obtained with this class of dyes is very wide (Gomes 2001). More than one azo group can be present in the dye structure, dyes then being classified as azo, disazo, trisazo or poliazo as they have one, two, three or more groups. The

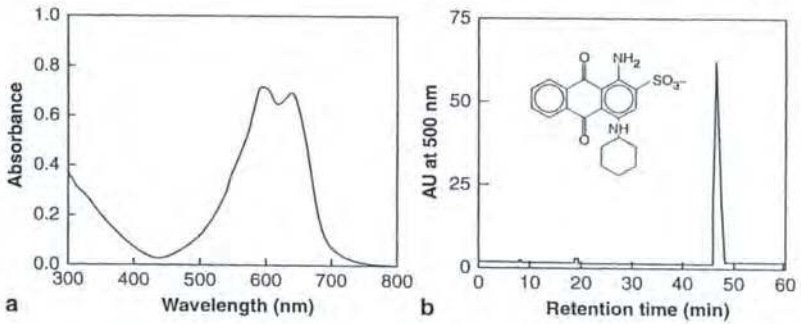

Fig. 4.2 Visible spectra (a) and HPLC chromatogram (b) of 1 mM Acid Blue 62 (Pereira et al. 2009b)

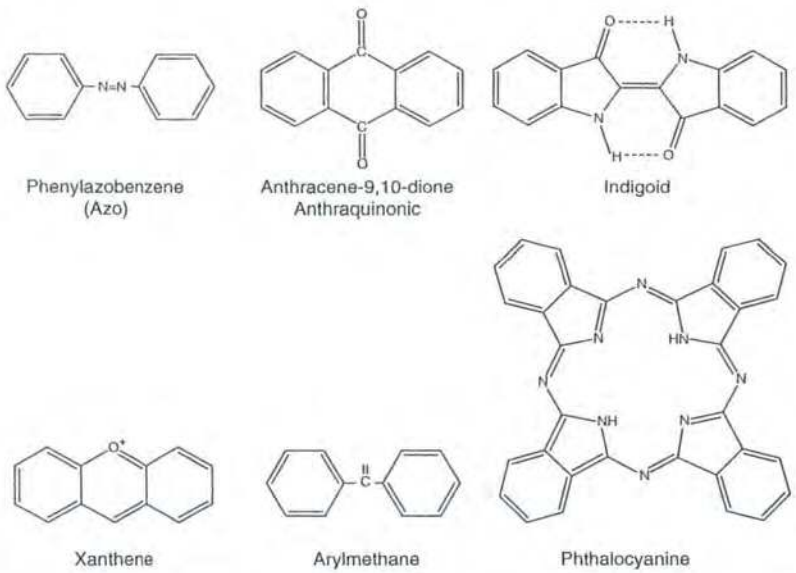

Fig. 4.3 Different chromophores: Azo, anthraquinonic, indigoid, xanthene, arylmethane and phthalocyanine dye structures

anthraquinonic dyes are also widely used and comprise of a carbonyl group associated with a conjugated system of 2 benzene rings. As with the azo dyes, substitution groups in the aromatic rings are required to intensify the colour. The major difference is their need only of electron donors once the carbonyl groups are in the uniquely possible position to act as electron acceptors.

Dyes are usually classified by their Colour Index (CI), developed by the Society of Dyes and Colourist (1984), which is edited every three months. It lists dyes firstly by a generic name based on its application and colour, then by assigning a 5-digit CI number based on its chemical structure, if known (O'Neill et al. 1999). Examples include Acid Blue 120 (26400), Reactive Red 4 (18105), and Mordant Yellow 10 (14010). They can be grouped in different classes: acid, basic, direct, disperse, metallic, mordant, pigment, reactive, solvent, sulphur and vat dyes, which reflects their macroscopic behaviour and also their prevailing functionalities. They are used in accordance to their compatibility with the type of textile substrate being processed (Gomes 2001). Acid, direct and reactive dyes are water-soluble anionic dyes; basic dyes are cationic, whereas disperse, pigment and solvent dyes are non-ionic (Hao et al. 2000; Gomes 2001). Disperse dyes are sparing soluble in water for application in hydrophobic fibres from a aqueous dispersion. They are often of anthraquinone and sulfide structure, with many –C=O, –NH– and aromatic groups (Fu and Viraraghavan 2001). Most of the mordant dyes are anionic, but some cationic ones also exist. In aqueous solution, anionic dyes carry a net charge due to the presence of sulphonate

(SO^{3-}) groups, while cationic dyes carry a net positive charge due to protonated amine or sulfur containing groups. Sulphonic groups in the molecule provide solubility. Disperse Vat dyes (of which indigo and woad are the most important examples) are water-insoluble; however, under reducing conditions, they can be converted into a 'leuco' form (soluble in alkaline aqueous solutions), which penetrates the fibres during dyeing. Metal-complex dyes exhibit higher light and wash fastness due to the presence of transition metals, such as chromium, copper, nickel or cobalt that modify the surface chemistry between the dye molecule and the fabric (Hao et al. 2000; Gomes 2001).

4.3 Dye Applications

Approximately 40 000 different synthetic dyes and pigments are used industrially, and about 450 000 tons of dyestuffs are produced worldwide. Azo dyes are the largest and more versatile class of dyes, accounting for up to 50% of the annual production (Zollinger 1987). They are extensively used in many fields of up-to-date technology, in e.g., various branches of the textile industry, the leather tanning industry, paper production, food, colour photography, pharmaceuticals and medicine, cosmetic, hair colourings, wood staining, agricultural, biological and chemical research, light-harvesting arrays, and photoelectrochemical cells (Kuhad et al. 2004; Couto 2009). Moreover, synthetic dyes have been employed for the efficacious control of sewage and wastewater treatment, for the determination of specific surface area of activated sludge for ground water tracing, etc. (Forgacs et al. 2004).

The largest consumer of these dyes is the textile industry, accounting for 2/3rds of its market. Different classes of dyes are used according to the fibres to which they can be applied. Reactive dyes are most commonly used as they can be applied to both in natural (wool, cotton, silk) and synthetic (modified acrylics) fibres (O'Neill et al. 1999). Reactive dyes differ from other class of dyes in that their molecules contain one or more reactive groups capable of forming a covalent bond with a compatible fibre group. They have become very popular due to their high wet-fastness, brilliance and range of hues (Hao et al. 2000). Their use has increased as synthetic fibres became more abundant. Acid and basic dyes are used for dyeing all natural fibres (wool, cotton, silk) and some synthetics (polyesters, acrylic and rayon). Direct dyes are classified this way because they are applied directly to cellulose fibres. Furthermore, they are used for colouring rayon, paper, leather and to a small extent nylon. The application of mordant dyes is limited to the colouring of wool, leather, furs and anodised aluminium. Solvent dyes are used for colouring inks, plastics, and wax, fat and mineral oil products.

4.4 Environmental Impact

Colour is usually the first contaminant to be recognized in a wastewater because a very small amount of synthetic dyes in water (< 1 ppm) are highly visible, affecting the aesthetic merit, transparency and gas solubility of water bodies. They adsorb

Table 4.3 Colour concentrations limits and quantum of water generated from industries (adapted from Anjaneyulu et al. 2005)

Industry	Quantum of water generated standards (m^3/Ton)	Colour concentration (hazen units)	Colour limits (hazen units)	
			USPHS	BIS
Textile	120 m^3/Ton_{fibre}	1100–1300	0–25	20
Pulp & Paper				
• Large	175 m^3/Ton_{paper}	100–600	0–10	5–101
• Small	150 m^3/Ton_{paper}			
Tannery	28 $m^3/Ton_{raw\ hide}$	400–500	10–50	25
Kraft mill	40 m^3/Ton	2100–2300	10–40	20
Sugar	0.4 m^3/Ton_{cane}	150–200	5–10	20

and reflect the sunlight entering water, thereby interfering with the aquatic species growth and hindering photosynthesis. Additionally, they can have acute and/or chronic effects on organisms depending on their concentration and length of exposure. Removal of colour from dye-containing wastewater is the first and major concern, but the point of degrading dyes is not only to remove colour, but to eliminate, or substantially decrease, the toxicity (i.e. detoxification).

Government legislation regarding the removal of dyes from industrial effluents is becoming increasingly stringent, especially in the more developed and developing countries (Robinson et al. 2001). Enforcement of the law will continue to ensure that textile and other dye-utilizing industries treat their dye-containing effluent to the required standards. In India, colour limits in industrial waters have also been set and have been made more stringent in the last few years. Table 4.3 presents the colour concentrations, their limits and the quantity of water generated from textile and other industries in United States and India (Anjaneyulu et al. 2005). European Community (EC) regulations are also becoming more stringent (O'Neill et al. 1999). A large variety of dyes can be found in real effluents. It has been estimated that ~9% (or 40 000 tons) of the total amount (450 000 tons) of dyestuffs produced in the world are discharged in textile wastewaters (O'Neill et al. 1999). Desirable criteria when producing those dyes are their fixation degree to fibre and fastness (i.e. high stability in light and washing) and resistant to microbial attack. Indeed, dyes are design to resist to very harsh conditions, difficulting colour removal from textile wastewaters by the conventional wastewater treatments. The degree of fixation of an individual dye varies with the type of fibre, shade and dyeing parameters. Dye fixation rate values are useful in giving an idea of the amount released, but can only be approximated. These losses are <2–10% for basic, disperse and direct dyes, but can reach 50% for reactive dyes (Al-Degs et al. 2000; Hao et al. 2000). This high degree for reactive dyes is due to the hydrolyzed form of reactive dyes which has no affinity for the fibres (Fig. 4.4). As nowadays, reactive dyes are the most commonly used in the textile industries, and there is a need for finding an efficient method dye removal with special attention to this class. Moreover, once in the effluents and due to their high stability, they may remain in the environment for a long time (~50 years). Because of their commercial importance, the impact and toxicity of dyes released in the environment have been extensively studied (Pinheiro et al.

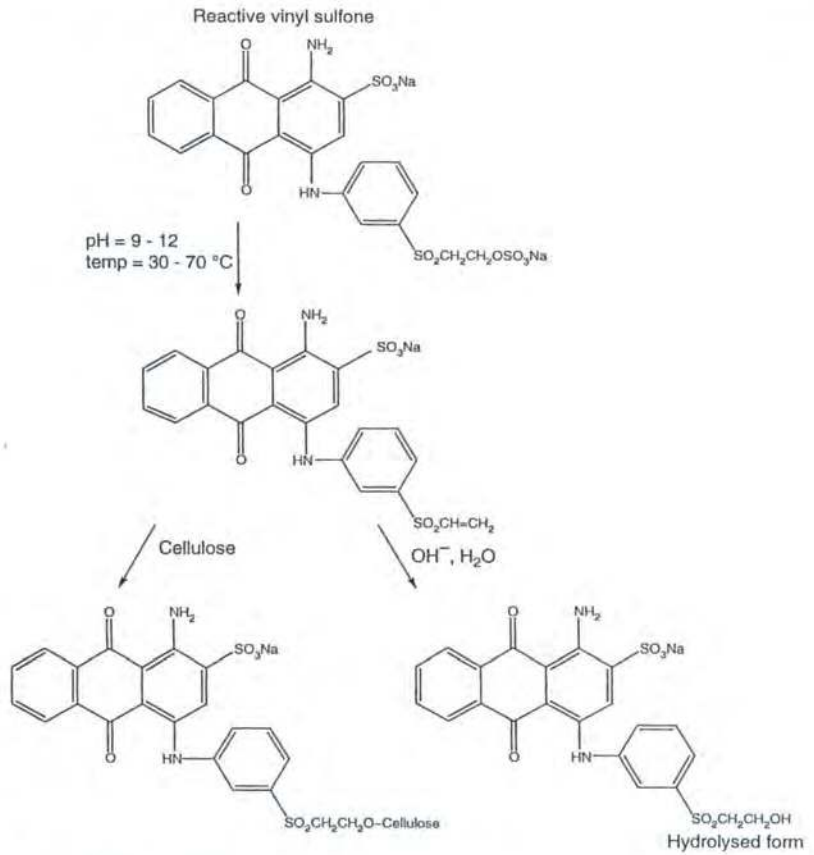

Fig. 4.4 Reactive dye undergoing hydrolysis (Hao et al. 2000)

2004; Mathur et al. 2003; Puvaneswari et al. 2006; Pereira et al. 2009a, b). As several thousand different synthetic dyes are employed, exhibit various biological activities, it is understandable that our knowledge concerning their behaviour in the environment and health hazards involved in their use, remain incomplete (Forgacs et al. 2004). In general, dyes have low toxicity in mammals and aquatic organisms (O'Neill et al. 1999), but products formed by their biodegradation, mainly aromatic amines from the anaerobic reduction of azo dyes (see "Dye biodegradation" section), can be harmful (Razo-Flores et al. 1997; Pinheiro et al. 2004). Definitely, azo dyes that constitute the largest group of synthetic colorants used are consequently the most common synthetic dyes released into the environment (Zhao and Hardin 2007; Ali 2010). Some have been linked to bladder cancer, splenic sarcomas,

and hepatocarcinomas, producing nuclear anomalies in experimental animals and chromosomal aberrations in cultured mammalian cells. An increased incidence of bladder cancer in dye workers exposed to large quantities of azo dyes has been reported (Puvaneswari et al. 2006). Assessment of the toxicity of dyes is therefore of the utmost importance. Various short-term screening methods have been developed to detect mutagenic/carcinogenic substances; these have played important roles not only in screening suspected chemicals, but also in studying the mechanisms of mutagenesis and carcinogenesis, thereby providing useful information for assessing the genetic effects of chemicals in man. Microorganisms have several attributes that make them attractive for use in quick screening of effluents and chemicals for toxicity. In the review of Hao et al. (2000) results on the toxicity valuation for the single cell alga, *Selenastrum capricornutum,* and for the fathead minnow, *Pimephales promelas,* are tabled. Other examples include the effect of the azo dye Sudan Orange G and the anthraquinonic dye Acid Blue 62 before and after an enzymatic treatment on the yeast *Saccharomyces cerevisiae* (Pereira et al. 2009a, b). The Ames test is a common, well implemented method for the evaluation of mutagenic potential of many compounds (Ames et al. 1975). The mutagenic potential of the locally (Indian) available and used textile dyes has been evaluated by Mathur et al. (2003) by the Ames test using the TA 100 strain of *Salmonella typhimurium*. Among the seven dyes tested, only one showed absence of mutagenic activity. The remaining six dyes were all positively mutagenic.

Also of particular concern are more specific compounds, used throughout the wet-processing steps, that can be toxic to aquatic life. Those include heavy metals, surfactants (wetting agents), fabric rinsing and/or washing detergents and other additives such as salts, sodium sulphate, sulphuric acid and dispersive agents). Additionally, dyeing baths often use extreme pH values (either acidic or alkaline, depending on the dye) and high temperatures; they have high BOD and chemical oxygen demand (COD), solid, oil and possibly toxic organics that include phenols (Shrestha and Kazama 2007; Tüfekci et al. 2007). These compounds will change the effluent water, causing a variety of physiological and biochemical disturbance. It is noteworthy that each fibre being processed produces effluents of its own distinctive characteristics, and for all textiles mill processing the same fibre, effluent characteristics are broadly similar, although quantities will vary. Differences can also arise between different plants processing the same fibre due to variations in the production technology. The impact of dyeing factories on plants and fishes can be found in the review of Puvaneswari et al. (2006).

Pollution prevention programs also need to focus on reduction in water and energy consumption by introducing new technologies and the reuse of dyeing water after the treatment processes. The textile industry is a high consumer of water (Allegre et al. 2004), with an average of 200 L water per kg of fibre. Another case of colour pollution is so-called "red-water", which results from trinitrotoluene (TNT) production generated in the purification stage (Hao et al. 1994). Other coloured wastewaters result from other industrial processes, including the bleaching of pulp, paper and textile fibres.

4.5 Wastewater Remediation

The textile finishing industry has been put under immense pressure to reduce use of harmful substances, especially mutagenic, carcinogenic, and allergenic effects of textile chemicals and textile dyes. There are regulations regarding the colour limits in effluents, which vary in different countries.

Textile dye wastewater remediation is based not only in colour removal (decolourisation), but also in the degradation and mineralization of the dye molecules. Indeed, decolourisation occurs when the molecules are removed from the solution or when the chromophore bond is broken, but the molecule in the first case, and the major fragments in the second, remain intact. The absorption of light by the associated molecules shifts from the visible to the ultraviolet or infrared region of the electromagnetic spectrum.

A wide range of technologies has been developed for the removal of synthetic dyes from waters and wastewaters to decrease their environmental impact. These include: **physical methods** such as membrane-filtration processes (nanofiltration, reverse osmosis, electrodialysis) and sorption techniques; **chemical methods** such as coagulation or flocculation combined with flotation and filtration, precipitation-flocculation with $Fe(II)/Ca(OH)_2$, electroflotation, electrokinetic coagulation, conventional oxidation methods (e.g. with ozone), irradiation or electrochemical processes; and **biological methods**, aerobic and anaerobic microbial degradation, and the use of pure enzymes. All of these procedures have advantages and disadvantages.

Traditional wastewater treatment technologies are markedly ineffective for handling wastewater of synthetic textile dyes because of the chemical stability of the pollutants (Forgacs et al. 2004). Additionally, they do not address the water recycling issue (Soares et al. 2004). The major disadvantage of physicochemical methods is primarily the high cost, low efficiency, limited versatility, need for specialized equipment, interference by other wastewater constituents, and the handling of the generated waste (van der Zee and Villaverde 2005; Kaushik and Malik 2009). Physical methods can effectively remove colour, but the dye molecules are not degraded, becoming concentrated and requiring proper disposal. With the chemical techniques, although the dyes are removed, accumulation of concentrated sludge can create a disposal problem. There is also the possibility that a secondary pollution problem arises because of the excessive amounts of chemicals involved. Recently, other emerging techniques—advanced oxidation processes, which are based on the generation of very powerful oxidizing agents such as hydroxyl radicals—have been applied with success in pollutant degradation (Arslan and Balcioğlu 1999; Zhou and He 2007). Although these methods are efficient for the treatment of waters contaminated with pollutants, they are very costly and commercially unattractive. The high electrical energy demand and the consumption of chemical reagents are common problems. The development of efficient, economic and environmentally friendly technologies to decrease dye content in wastewater to acceptable levels at affordable cost is of utmost importance (Couto 2009). Biological methods are generally considered environmentally friendly because they can lead to complete mineraliza-

tion of organic pollutants at low cost (Pandey et al. 2007). They also remove BOD, COD and suspended solids. The main limitation can be related in some cases to the toxicity of some dyes and/or their degradation products to the organisms used in the process. Indeed, the removal of dyes depends on their physical and chemical characteristics, as well as the selected treatment method, with no technology in use today having universal application. However, some of the processes do not satisfactorily remove the colour and others are costly (Mondal 2008). The technologies used and those in development for the dye removal have been discussed in several reports (Vandevivere et al. 1998; Robinson et al. 2001; Forgacs et al. 2004; Anjaneyulu et al. 2005; Hai et al. 2007; Hao et al. 2007; Mondal 2008).

4.5.1 Sorption

Sorption of synthetic dyes on inexpensive and efficient solid supports has been considered a simple and economical method for their removal from water and wastewater, producing high quality of water (Forgacs et al. 2004; Allen and Koumanova 2005), making it an attractive alternative for the treatment of contaminated waters, especially where the sorbent is inexpensive and does not require a pre-treatment step before its application. It is superior to other techniques for water re-use in terms of initial cost, flexibility and simplicity of design, ease of operation, lower interference with diurnal variation, and insensitivity to toxic pollutants. Some of the advantages of applying sorption (see Weber et al. 1978; Mckay et al. 1999; Amin 2008) include: (1) less land area (half to quarter of what is required for a biological system); (2) lower sensitivity to diurnal variation; (3) not being affected by toxic chemicals; (4) greater flexibility in design and operation, and (5) superior removal of organic contaminants. Furthermore sorption does not result in the formation of harmful substances.

Decolourisation by sorption is a result of two mechanisms, sorption and ion exchange, and is influenced by many physicochemical factors, such as, dye/sorbent interaction, sorbent surface area, particle size, temperature, pH, and contact time (Robinson et al. 2001; Dhodapkar et al. 2006; Jovančić and Radetić 2008). The nature of the bound between the dye and the adsorbent during sorption is important for its effectiveness. Two types of sorption occur: **physical sorption**, when the interparticle bonds between the adsorbate and adsorbent are weak (van der Waals, hydrogen, and dipole-dipole); and **chemical sorption**, characterized by strong interparticle bonds due to an exchange of electrons (covalent and ionic bonds). The first is usually a reversible process and the second irreversible (Allen and Koumanova 2005).

An effective sorption model requires an accurate equilibrium isotherm, kinetic/mass transfer relationships and coupling equations (Mckay 1998). The mass transfer stage usually assumes a three-step model: (i) external film diffusion across the boundary layer, (ii) sorption at a surface site, and (iii) internal mass transfer within the particle, based on a pore or solid surface diffusion mechanism (Mckay and Sweeney 1980). To explain the sorption mechanism and predict sorption uptake rates by adsorbent pellets, various assumptions have been applied to equilibrium data: linear isotherm (Dryden and Kay 1954), irreversible isotherm (Liapis 1987) and nonlinear isotherm (Tien 1961; Weber and Rummer 1965). Specific cases of nonlinear isotherms include the Langmuir equation (Langmuir 1918), the Freundlich equation (Freundlich 1906) and the Redlich-Peterson equation (Redlich and Peterson 1959). The **Langmuir and Freundlich sorption isotherms** (Eqs. 4.1 and 4.2, respectively) are the most commonly used to quantify the amount of adsorbate adsorbed by an adsorbent (Annadurai and Krishnan 1997; Al-Degs et al. 2000, 2008).

$$q_e = Q_{max} K_L C_e/(1 + K_L C_e) \quad \text{(Langmuir)} \quad (1)$$

$$q_e = K_F C_e^n \quad \text{(Freundlich)} \quad (2)$$

Where, q_e (mmol·g_{AC}^{-1}) is the surface concentration of dye at equlibrium; C_e (mmol·dm^3) is the equilibrium concentration of dye in solution; Q_{max} (mmol·g_{AC}^{-1}) is the amount of dye adsorbed at a complete monolayer coverage; K_L (dm^3.$mmol^{-1}$) is a constant that relates to the heat of sorption; K_F [mmol·g^{-1}(mmol·dm^3)$^{-n}$] represents the sorption capacity when the dye equilibrium concentration (C_e) equals one unit, and **n** represents the degree of dependence of sorption on the equilibrium concentration.

Sorption is also related to the molecular size of the dye and the number of sulfonic groups. Smaller dyes have higher sorption, whereas bigger molecules are more difficult to adsorb due to diffusion limitations (Allen and Koumanova 2005; Tsang et al. 2007).

Sorption methods, independently of the inorganic or organic character of the supports, have certain drawbacks. Since sorption processes are generally non-selective, other components of the wastewater can also be adsorbed by the support. Competition between the sorbents can influence the dye-binding capacity of supports in an unpredictable manner. Moreover, a sorption process removes the synthetic dyes from wastewater by concentrating them on the surface, without structurally changing them. When the support is regenerated, the fate of the resulting concentrated solution of dyes presents a problem that has not yet been satisfactorily solved.

Large-scale applications based on the sorption process have to take all these factors into consideration. This technique can be successfully applied as a polishing step at the end of the treatment stage, to meet discharge colour standards. The reviews of Allen and Koumanova (2005) and Mondal (2008) outline the fundamental principles of dye sorption and also evaluate a number of different adsorbents used in the removal of colorants from wastewaters.

4.5.1.1 Activated Carbon

Activated Carbon (AC) is the most widely used sorbent and therefore will be described in more detail, but some of the fundamental principles outlined are also applicable to other adsorbents.

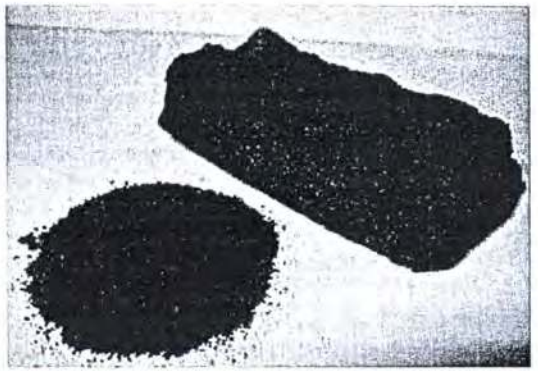

Fig. 4.5 Image of Activated Carbon both in powder and block form (AC)

Commercial ACs are available (Fig. 4.5), with different physical forms depending on their application. The type of carbon sorbent and its mode of preparation exert a marked influence on the sorption capacity (Pereira et al. 2003; Forgacs et al. 2004), and both by their texture and surface chemistry determine the performance (Figueiredo et al. 1999). The diversity of surface groups on AC (of acid and base character) makes it much more versatile than other adsorbents (Fig. 4.6) and the nature and concentration of surface functional groups can be modified by suitable chemical or thermal treatments. It is also possible to prepare carbons with different proportions of micro, meso and macropores (Rodriguez-Reinoso 1998; Figueiredo et al. 1999). This becomes advantageous once AC is modified physical and chemically, for specific applications, in order to optimise its performance. Liquid (HNO_3) and gas oxidation (O_2) produce samples with a higher amount of surface oxygen-containing groups. Nitric treatment increases in particular the carboxylic acid groups and the gas oxidation treatment is the most effective way to introduce less acidic ones, such as phenols and carbonyl/quinone groups (Figueiredo et al. 1999; Pereira et al. 2003; Faria et al. 2005). No significant changes in the texture of the carbon are likely to occur with liquid phase treatments, while an increase of the micropore volume, mesopore surface area and average of the micropores with gas phase oxidation is usual; these changes increase as the degree of oxidation itself increases (Figueiredo et al. 1999). Thermal treatment at high temperature produce materials with a low amount of oxygen-containing groups and high basicity, resulting mainly from the ketonic groups remaining on the surface, fewer acidic groups, and the delocalisation of π-electrons of the carbon basal planes (Moreno-Castilha et al. 2000; Faria et al. 2005, 2008).

Activated carbon samples are amphoteric in nature and therefore the pH of a dye solution plays an important role in the sorption process. As already mentioned, acidity and basicity is related to the chemical groups at the AC surface. The charge thereon is correlated with the pH of the solution. A convenient index of the propensity of a surface to became either positively or negatively charged depends on the pH required to give a zero net surface charge (pH_{pzc}). In a solution at $pH<pH_{pzc}$,

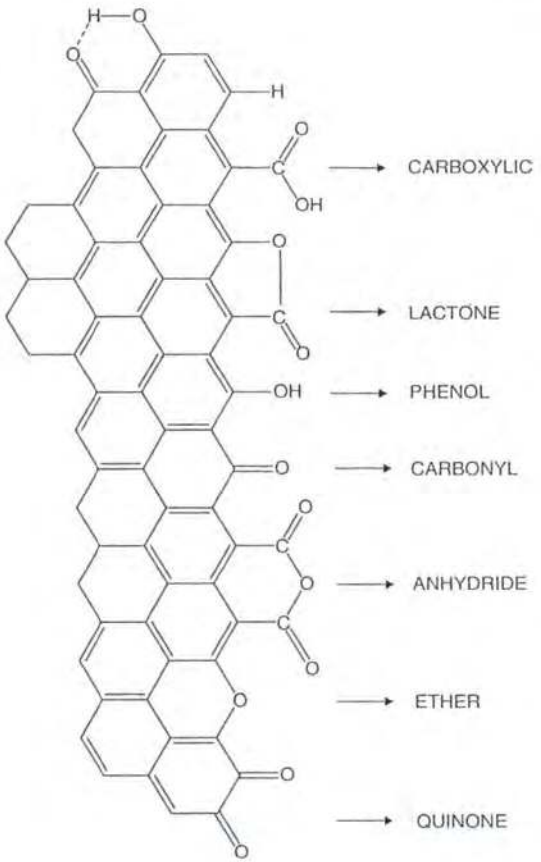

Fig. 4.6 Possible surface groups at carbon surface (adapted from Figueiredo et al. 1999)

AC has a net positive surface charge and at $pH>pH_{pzc}$ a net negative charge. High sorption on AC is expected at high pH for cationic dyes and at low pH for anionic dyes, due to electrostatic interaction and attractive forces (**Coulomb's law**). In contrast, when the pH of the solution is not in the ideal range, the electrostatic repulsive forces repel the dyes from the AC surface and it will be covered by the solvent. Some experimental results also indicate that sorption capacity increases with a decrease in AC particle size (Mckay and Sweeney 1980; Al-Degs et al. 2000). This can be attributed to the large molecular diameter and chemical nature of the adsorbate species. Decolourisation is also dependent of other parameters, such as the molecular structure, pK_a and redox potential of the dye. Dye ionization (protonation/deprotonation) is also dependent on the pH of the solution. A variety of experimental techniques have been used to characterize the AC surfaces, including

chemical titration, temperature-programmed desorption (TPD), X-ray photoelectron spectroscopy (XPS), mass spectrometry, NMR, infra-red spectroscopy (FTIR, DRIFTS) (Rodriguez-Reinoso 1987; Figueiredo et al. 1999; Pereira et al. 2003; Faria et al. 2004; Shen et al. 2008; Klein et al. 2008).

Carbon-based sorbents have excellent sorption properties for a considerable number of synthetic dyes (Pereira et al. 2003; Malik 2004; Faria et al. 2005, 2008; Al-Degs et al. 2008). The possibility of using of AC as a redox mediator in dye biological degradation has more recently, also been proposed (van der Zee et al. 2001; Pereira et al. 2010; see also Sect. 4.5.6.4). However, preparation of carbon sorbents is generally energy-consuming, making commercially available products relatively expensive. Since a large amount of carbon sorbent is needed for the removal of dyes from a large volume of effluent, the high cost can hamper its application (Fu and Viraraghavan 2001; Forgacs et al. 2004). In addition, the technology for manufacturing AC of good quality is not fully in place in developing countries. This has prompted a growing research interest in the production of low-cost alternatives to AC from a range of carbonaceous and mineral precursors.

4.5.1.2 Other Adsorbents

Research to find cheaper alternatives to AC has been discussed by Gupta and Suhas (2009). Sorption capacity and selectivity are also factors important when choosing the material for certain applications. Many materials have proved to be good candidates, including inorganic and organic materials, some examples with their advantages and disadvantages being given in Table 4.4. Many of the starting materials for these replacement adsorbents are agricultural or industrial by-products; hence their reuse as secondary adsorbents minimizes waste. But applicability of the sorption process is largely dependent on the availability of cheap adsorbents and thus recent initiatives in sorption process have sought economically sound adsorbents.

4.5.1.3 Biosorption/Biomaterials

Biosorption is defined as the accumulation and concentration of pollutants from aqueous solutions using biological materials, thus allowing the recovery and/or environmentally acceptable disposal of pollutants. Biosorption, a property of both living and dead organisms (and their components), has been heralded for a number of years as a promising biotechnology for pollutant removal from solution and/or pollutant recovery, because of its efficiency and simplicity, as an analogous operation to conventional ion-exchange technology, and the availability of biomass (Aksu 2005; Gadd 2009). Biosorption for dyes can also be adopted for the treatment of textile effluents since a wide variety of microorganisms including algae, yeasts, bacteria and fungi are capable of efficiently decolourising a huge range of dyes (Fu and Viraraghavan 2001; Padmesh et al. 2005; Prigione et al. 2008a, b; Bergsten-

Table 4.4 Example of dye adsorbents, major advantages and disadvantages of their use

Adsorbent	Advantages	Disadvantages	References
Alumina	• can be modified in order to be improved as sorbent • can be regenerated • has high affinity for cationic dyes	• has lower affinity for anionic dyes	Adak et al. (2005), Adak and Pal (2006)
Coal (high silica content)	• achieve equilibrium in short time as compared with activated carbon • is effective for cationic dyes (basic dyes) due to the low pH_{pzc} • not being a pure material is suggested to have a variety of surface properties and sorption properties	• not effective for anionic dyes (acid, direct and reactive), which are the most commonly use and highly released to wastewaters;	Mohan et al. (2002), Karaca et al. (2004)
Fly ash	• high availability • after its use in construction, the safe disposal of this material is problematic; its use on sorption will solve also this environmental problem • low cost material • has hydrophilic surface and porous structure • remove both anionic and cationic dyes • inexpensive by-product management technology is needed for its re-use	• some fly ash have unstable composition and properties (depending on their origin) • can contain high level of toxic metals	Mohan et al. (2002), Wang and Wu (2006)
Silica-based and clay (high silica content) $pH_{pzc} \sim 2$	• low cost • available in abundance • good sorption properties	• not effective for the anionic dyes (acid, direct and reactive), which are the most commonly use and highly released to wastewaters • side reactions	Kannan et al. (2008)
Diatomite (siliceous rock from the skeletons of aquatic plants, diatoms); $pH_{pzc} = 6.2$	• presence of high reactive groups (silanol); • it has a unique combination of physical and chemical properties, which make it applicable as a sorbent; • high selectivity for basic and reactive dyes; • high permeability • high porosity, low density and high surface area	• low selectivity for anionic dyes • naturally occurring diatomice has a lesser ability to adsorb dyes compared with the chemically modified diatomice	Al-Ghouti et al. (2003), Allen and Koumanova (2005), Badii et al. (2010)

Table 4.4 (continued)

Adsorbent	Advantages	Disadvantages	References
Functional granular polymers	• high selectivity for the removal of cationic dyes • exhibits relatively high surface area and porosity • its ease of regeneration	• solubility in water can be low • high cost	Chowdhury et al. (2004)
Agricultural, industrial and domestic waste by-products	• widespread availability • economically attractive for dye removal • because they are so cheap, regeneration is not necessary	• usually can be used just once	Nigam et al. (2000), Forgacs et al. (2004)
Peat (low-grade carbonaceous fuel containing lignin, cellulose and humic acids)	• widely available biosorbent • costs much less than activated carbon • has adsorption capabilities for a variety of pollutants • good adsorption for cationic dyes (basic) at high pH • does nor requires activation • the exhausted peat adsorbent may be disposed of by burning and the heat used or steam generation • can also be modified with some chemical pretreatment to improve its sorption properties and selectivity	• low mechanical strength • high affinity for water • poor chemical stability • tendency to shrink and/or swell, and to leach fulvic acid • influenced by the pH of solution • low capacity for acid dyes (anionic); • specific surface area for adsorption is lower than that of activated carbon	Brown et al. (2000)
Zeolite (mezoporous material)	• natural zeolites have excellent ion exchange properties and high surface area • well defined pore structure in the microporous range • surface can be modified by quaternary amine surfactants enhancing the adsorption capacity	• smaller dyes (such many azo) are excluded from zeolite structure due to the large pore size; • natural zeolites are not suitable sorbents for reactive azo dyes	Alpat et al. (2008), Armagan et al. (2004), Ozdemir et al. (2004)

Torralba et al. 2009). Although the mechanisms of biosorption are not yet fully explained, it seems to take place essentially on the cell wall.

Textile dyes vary greatly in their chemistries and, therefore, their interactions with microorganisms/biomaterials depends on a particular dye and also on the specific chemistry of microbial biomass/biomaterial (Robinson et al. 2001; Erdem et al. 2005). The use of biomass for wastewater treatment is increasing because it is available in large quantities at a low price. The major advantages of biosorption technology are its effectiveness in reducing the concentration of dyes down to very low levels, and the use of cheap biosorbent material. Fungal biomass can be produced economically using relatively simple fermentation techniques and cheap growth media (Fu and Viraraghavan 2002). Generally, it can be eluted and regenerated by some solvents, such as methanol and ethanol, certain surfactants (e.g. non-ionic Tween), or NaOH solution. Biosorption as an emerging technology also attempts to overcome the selectivity disadvantage of conventional sorption processes. The use of dead rather than live biomass eliminates the problems of toxic waste and nutrient requirements. In spite of good sorption properties and high selectivity, the sorption process is slow. Clogging in some bioreactors has also been a limitation. Biosorption processes are particularly suitable for the treatment of solutions containing dilute (toxic) dye concentrations. Biomass has a high potential as a sorbent because of its physico-chemical characteristics. Biosorption is strongly influenced by the functional groups in the fungal biomass, the specific surface properties, and the initial pH of the dye solution. Its performance also depends on external factors such as salts and ions in solution that may be competing with the dye.

Not only microorganisms, but other biomaterials are being increasingly used for economical and ecofriendly remediation of textile dye from effluents (Crini 2006; Allen and Koumanova 2005; Mondal 2008). **Chitin/Chitosan**-based absorbents present a new group of bioabsorbents that can remove dyes from wastewater (Chiou and Li 2002; Chatterjee et al. 2005). **Chitin** is a naturally occurring derivative of cellulose and **chitosan** is a dervative of chitin. Chitosan is the deacylated form of chitin, which is a linear polymer of acylamino-D-glucose. Chitosan has the attraction of having a high content of amino and hydroxyl functional groups, giving it a high potential for dye sorption (Yoshida et al. 1991, 1993; Juang et al. 1997; Chiou and Li 2002). These materials can contain amine or amide nitrogen in varying proportions, and have high sorption capacity for anionic dyes without significant sorption for cationic dyes. Van der Walls attraction, hydrogen bonding and Coulombic attraction are the main ones involved. Sorption capacity of chitosan increases with a decrease in pH: the $-NH_2$ group is easily protonated in acid solution to become NH_3^+, thereby creating electrostatic attraction to anionic dyes. Chitin contains the amide group –CO–NH– which cannot easily be protonated, and hence less electrostatic attraction between the chitin and the dye molecules occurs. Some of the useful features of chitosan include its abundance (it is found in the skins or shells of anthropods), hydrophilicity, biodegradability and nontoxicity.

4.5.2 Coagulation-Flocculation-Precipitation

Coagulation is the destabilization of electrostatic interactions that exist between the molecules of reactive hydrolyzed dyes (or auxiliaries) and water through the addition of a chemical reagent, a coagulant (Allegre et al. 2004). Coagulation is used together with flocculation or sedimentation, with an efficiency that depends on the type of flocculant and the pH of the medium (Allegre et al. 2004; Mondal 2008). In practice, flocculation reagents are large synthetic polymers with a linear structure used along with $FeCl_3$, $FeSO_4$, $AlCl_3$ or $Al_2(SO_4)_3$. There have been reports of the use of several co-polymers, such as pentaethylene, hexamine and ethylediene dichloride, as flocculants for the decolourizing of dye effluents (Anjaneyulu et al. 2005). Colour removal is accomplished by aggregation/precipitation and sorption of colouring substances onto the polynuclear coagulant species and hydrated flocks. The co-polymers have large pore diameters (up to ~400 μm), thereby enhancing the process of sorption on flocs.

Although these methods are advantageous in eliminating insoluble dyes (such as disperse), a large amount of sludge is produced leading to extra costs for its treatment (Hao et al. 2000). Acid, direct, vat, mordant and reactive dyes usually coagulate, but the resulting floc is of poor quality and does not settle well, yielding mediocre results. Cationic dyes simply do not coagulate.

Some features of the coagulation process include (Soares et al. 2004):

- Short detention times;
- Good removal efficiencies;
- Quick response to operational factors, making automation simple;
- Equipment requiring less space than a biological lagoon (but nevertheless expensive);
- Removal of 90% of suspended solids and ~10–50% COD;
- Performance dependent on the final floc formation and its setting quality
- Overdoses of polyelectrolyte leading to residual concentrations of it in the effluent, and thus a detrimental effect on the nitrification process;
- Dye colour in solid phase remaining a problem, although removal from the aqueous phase can be up to 90%;
- Sludge production dependent on the nature of the flocculant used

The high cost of chemicals for precipitation and pH adjustments, problems associated with dewatering and disposing of generated sludge, and the high concentration of residual cation levels left in the supernatant are some of the limitations of this method.

4.5.3 Membrane Filtration

Membrane filtration is another important process for separating dyes from aqueous solution. A membrane is a permeable or semi-permeable phase, often known as a

thin polymeric solid, which restricts the motion of certain species. A membrane is a barrier that allows one component of a mixture to permeate freely while hindering permeation of another component. Membrane techniques offer the appeal of recovering and reusing chemicals (dyes) for producing reusable water. This separation process has the ability to clarify, concentrate and, most importantly, to separate dye continuously from effluent. The technique effectively removes of all type of dyes, but produces highly concentrated sludge. Dissolved solids are not removed by this technique. Selection of a specific membrane depends on the e type of dye or wastewater composition, and on the process operation. Membranes with varying pore size can retain solutes according to their different molecular weight cutoffs (MWCO) and are classified as: **reverse osmosis** (<1000 MWCO), **nanofiltration** (500–15 000 MWCO) and **ultrafiltration** membranes (1000–100 000 MWCO). [Nanofiltration (NF) membranes are a new class of membranes, which have properties between those of ultrafiltration (UF), and reverse osmosis (RO) membranes.]

The major drawbacks are the high costs of labour and of membrane replacement, since membranes are prone to clogging and fouling. The need to be regenerated or changed at regular intervals, which entails high capital and energy costs, and this is not always technical and economically viable. Additionally, adsorbed dye molecules are not degraded but concentrated, and subsequently need to be disposed of. Membrane filtration is therefore more effective for low concentrated waters. Membrane blockage due to high dye concentration was observed by Ahmad et al. (2002). When the feed concentration of dye increased from 0.5 to 10 gL^{-1}, the flux decreased dramatically from 1904 to 208 L/m^2 h, and the average percentage rejection increased from 21 to 91%.

4.5.4 Electrochemical Wastewater Treatment

The electrochemical treatment of wastewater is a potential powerful method of pollution control, offering high removal efficiency. The process includes **electrooxidation** and **electrocoagulation,** which are relatively non-specific, and thus applicable to a variety of contaminants.

The principle of electrochemical techniques is to send an electric current through electrodes resulting in different chemical reactions (Fig. 4.7). The reducing agent, instead of being added in the typical conventional method is replaced by an innovative cathodic *electron transfer* (electrons are employed, instead of chemicals). An electrochemical cell is used to perform the reduction/oxidation process. The dyeing apparatus is coupled to the electrochemical cell and the dye bath gets effectively reduced through an electrochemical process. The factors affecting electrochemical performance include current intensity, design geometry, stratification, type, number and spacing of electrodes used, pH, temperature, nature of electrolyte, surface tension, flow rate of wastewater and the properties of the dye (Hao et al. 2000). Higher degrees of decolourisation can be achieved by this method, although this does not imply a high COD reduction. Depending on electrolysis time and the applied poten-

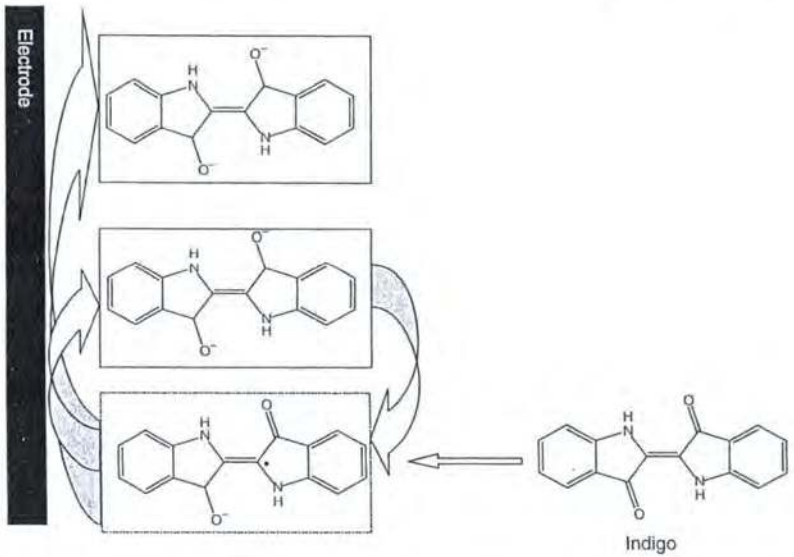

Fig. 4.7 Mechanism of the direct electrochemical reduction of indigo (Adapted from Roessler et al. 2001)

tial, total decolourisation can be attained. The electrochemical method of oxidation for colour removal is more efficient in the treatment of textile wastewater for the dyeing stage than for the total dyeing and finishing stages. Some of the advantages are non-hazardous resulting products, the little need for additional chemicals, and the low temperature required compared with other treatments (Kim et al. 2002b; Esteves and Silva 2004); furthermore no sludge is formed. In terms of apparatus, it does not require much space. Electrochemical processes generally have lower temperature requirements than those of other equivalent non-electrochemical treatments, and no need for additional chemicals. The equipment and operations needed are generally simple, with the controls being easy and the electrochemical reactors compact. The high cost of the electricity requirement is the main limitation (Kim et al. 2002a).

4.5.5 Advanced Oxidation Processes

Oxidation is the most commonly used chemical decolouration processes because it is simple. Alternative to the traditional methods are the Advanced Oxidation Processes (AOPs) based on the generation of highly reactive species, such as hydroxyl radicals (•OH), that can oxidize quickly and non-selectively a broad range of or-

ganic pollutants. Furthermore, several investigations have demonstrated that AOPs effectively remove colour and, in part, some organic content of dyestuffs. AOPs include (see Rauf and Ashraf 2009):

1. photolysis (UV);
2. hydrogen peroxide, such as H_2O_2+UV, Fenton ($H_2O_2+Fe^{2+}/Fe^{3+}$), Fenton-like reagents ($H_2O_2+Fe^{2+}$-solid/Fe^{3+}-solid) and photo-Fenton ($H_2O_2+Fe^{2+}/Fe^{3+}$+UV);
3. ozone (ozonation, photo-ozonation, ozonation + catalysis, and $O_3+H_2O_2$ and O_3+Fe^{2+}/Fe^{3+}); and
4. photocatalysis (semiconductor-mediated photocatalysis and TiO_2+CdS+combinations).

AOPs have common principles in terms of the participation of hydroxyl radicals generally assumed to participate in the reaction. Although other species are also thought to be involved, the active species responsible for the destruction of contaminants in most cases is the hydroxyl radical (•OH), which is unstable and therefore very reactive. Hydroxyl radicals may attack organic molecules by extracting a hydrogen atom from the molecule under attack (Hao et al. 2000). The common pathway for the degradation of organics by the hydroxyl radical is as follows:

$$\bullet OH + RH \rightarrow H_2O + R\bullet$$

$$R\bullet + H_2O_2 \rightarrow ROH + \bullet OH$$

$$R\bullet + O_2 \rightarrow ROO\bullet$$

$$ROO\bullet + RH \rightarrow ROOH + R\bullet$$

The oxidation potential of •OH is higher than that of other oxidizing agents (Table 4.5). Hydrogen peroxide (H_2O_2) is a widely used agent that needs to be activated by, for example, UV light. Many methods of chemical decolourisation vary depending on the method by which H_2O_2 becomes activated. H_2O_2 alone is often not effective and fails to react with a particular azo dye over a pH range of 3–9.5 (Hao et al. 2000). For light-mediated oxidation of dye molecules, UV of different wavelengths and visible light can be used. As expected, UV alone may not be applicable to wastewaters with high colour intensity. But the combination of UV and H_2O_2 can effectively decolourise dyes, and also reduce TOC and COD, and hence to a potential mineralization.

Table 4.5 Oxidation potential for several oxidizing agents (Hao et al. 2000)

Process	E_0 (V)
$O_2+4\,H^++2\,e^- \rightarrow 2\,H_2O$	1.23
$H_2O_2+2\,H^++2\,e^- \rightarrow 2\,H_2O$	1.78
$O_3+2\,H^++2\,e^- \rightarrow H_2O_2$	2.07
$\bullet OH+H^++e^- \rightarrow H_2O$	2.28

In Fenton's reactions, the combination of ferrous and H_2O_2 serves two functions: oxidation of dye molecules by •OH and coagulation with iron ions. After addition of the iron and hydrogen peroxide, they react together to generate hydroxyl radicals in the following manner:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \bullet OH + OH^-$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + \bullet OOH + OH^+$$

The generated hydroxyl radicals will in turn react with the pollutants and oxidize them. With the formation of Fe^{3+} and its complexes, the Fenton process can precipitate the dissolved solutes. Fenton's reagent is effective in decolourising both soluble and insoluble dyes, but leads to the formation of a sludge that can present disposal problems. The pH of wastewater needs to be acidified for effective utilization of Fenton's reagent; if it is is too high, the iron precipitates as $Fe(OH)_3$ and decomposes the H_2O_2 to oxygen. The use of ozone, pioneered in the early 1970s, is due its high instability, making it a good oxidizing agent. Ozonation, as an effective oxidation process, has found application in the decolourisation of synthetic dyes; although it is ineffective in dispersing dyes and needs high pH values. With no residual or sludge formation and no toxic metabolites, ozonation leads to uncoloured effluent and low COD, suitable for discharge into aqueous systems. One major advantage is that ozone can be used in its gaseous state and therefore does not increase the volume of wastewater and sludge. The disadvantage of ozonation is the short half-life (typically being 20 min) of ozone, demanding continuous application and thereby making it a costly process. Operating costs for ozone have proved higher than for electrochemical treatment giving the same level of colour removal.

Photocatalytic degradation is a part of AOP which has proven to be a promising technology for degrading organic compounds (Rauf and Ashraf 2009). Commercial dyes are designed to resist photodegradation, and hence the selection of optimal photocatalytic conditions for the decolourisation of dyes requires considerable expertise. Direct and indirect photocatalytic pathways are the 2 suggested mechanisms for a given photocatalytic reaction. The effective and economic performance of the process is strongly dependent on the electrode materials, and many researchers have investigated electrochemical oxidation for azo dye degradation through operating parameter optimization using a variety of anodes. With the advancement of experimental techniques, semiconductors have been tested for their efficiencies in dye degradation, including TiO_2, V_2O_5, ZnO, WO_3, CdS, ZrO_2 and their impregnated forms (Rauf and Ashraf 2009). Other factors affecting photochemical reactions include pH, light intensity, temperature and initial dye concentration.

Ultraviolet photolysis combined with hydrogen peroxide (UV/H_2O_2) is one of the most appropriate AOP technologies for removing toxic organics from water because it probably occurs in nature itself (Schrank et al. 2007). While the UV/H_2O_2 process appears to be very slow, costly and weakly effective for possible full-scale application, the combination of UV/TiO_2 is more promising (Dominguez et al. 2005). TiO_2 during photocatalysis generates electron hole pairs when irradiated by the light of <380 nm wavelength. Organic pollutants are thus oxidized via direct

hole transfer or, in most cases, attacked by the •OH radical formed in the irradiated TiO_2 (Xu 2001).

One of the disadvantages of AOP's is that they may also produce undesirable toxic products and release aromatic amines. In aqueous solution, photochemical degradation is likely to progress slowly, but there is no sludge production.

4.5.6 *Bioremediation*

Bioremediation is defined as the biologically mediated breakdown of chemical compounds: microorganisms (filamentous fungi, yeasts, bacteria, actinomycetes and algae) or enzymes are applied to assist in the removal of xenobiotics (synthetic organic compounds, which are not found in nature, and are thus foreign and new to the biota) from polluted environments. Biological processes are design to take advantage of the biochemical reactions that are carried out in living cells and/or via the enzymes synthesised by them. They are energy-dependent processes and usually involve the breakdown of the pollutant into a number of by-products.

Biodegradation of synthetic dyes has been identified as an effective and environmentally friendly solution (Fig. 4.8). Synthetic dyes are not commonly present in the environment, so they may not be readily biodegradable. Observations indicate that dyes themselves are not biologically degradable, since microorganisms do

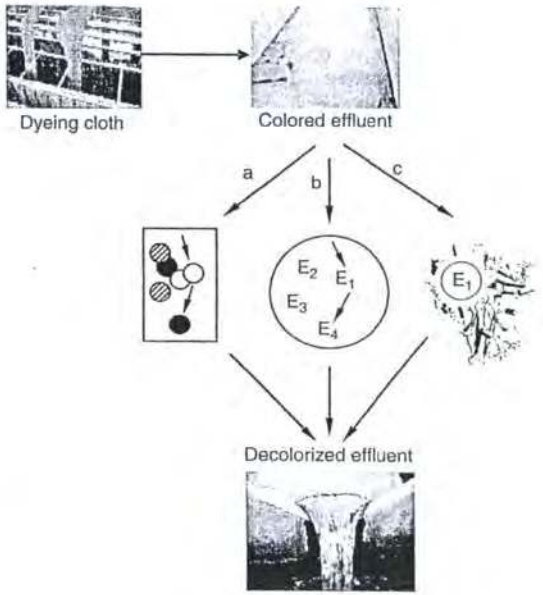

Fig. 4.8 Dye bioremediation by: (a) mixed cultures, (b) isolated organisms, and (c) isolated enzymes (e) (Adapted from Kandelbauer and Guebitz 2005)

not use colour constituents as a source of food. For bioremediation, an additional carbon and energy source has to be present (Nigam et al. 1996). In addition, synthetic dyes are designed in such a way that they can be made resistant to microbial degradation under aerobic conditions. Also the high solubility in water of, especially, sulfonate dyes and their high molecular weight inhibit permeation through biological membranes. Bio-decolourisation of dyes may occur either by sorption on growing/living and dead microbial cells, biosorption, or biodegradation of the dyes by the cells (Fu and Viraraghavan 2001). In biosorption, the original structure of the dyes remains intact and the environmental pollution problem is not eradicated because the pollutant is not destroyed but instead becomes entrapped in the microbial biomass (Ali 2010). In contrast, in biodegradation the original dye structure is destroyed, and the pollutant is split into fragments by the microbial cells, sometimes achieving complete mineralization, i.e., conversion of the xenobiotic into CO_2, H_2O and some salts of inorganic origin. Although many organic molecules are degraded, many others are recalcitrant due to their complex chemical structure, and to their synthetic origin and xenobiotic nature. The rates of dye decolourisation/biodegradation are usually assessed by Monod-type kinetics. Zero, first and second-order reaction kinetics have been used to estimate colour removal rate constants using Eqs. 4.3, 4.4 and 4.5, respectively:

$$C_t = C_0 - k_0 t \quad \text{(zero order)} \tag{3}$$

$$C_t = C_0 e^{-k1t} \quad \text{(first order)} \tag{4}$$

$$C_t = 1/C_0 + k_2 t \quad \text{(second order)} \tag{5}$$

where C_0 and C_t are the initial and time of reaction concentrations, respectively; t is the reaction time, and k is the reaction rate constant ($time^{-1}$).

Biological treatment is often the most economical alternative compared with other physical and chemical processes. An advantage of biological treatment over certain physicochemical treatment methods is that >70% of the organic material measured by the COD test can be converted to biosolids (Forgacs et al. 2004).

Development of an efficient dye degradation biotechnology requires application of a suitable selected strain and its use under favourable conditions to optimize its degradation potential (Novotný et al. 2004; Lucas et al. 2008). A number of microorganisms are capable of decolourising textile dyes, including bacteria, filamentous fungi and yeasts (Stolz 2001). The isolation of new strains or the adaptation of existing ones to the decomposition of dyes will probably increase the efficacy of bioremediation of dyes in the near future. The addition of activators (e.g. Tween 80, veratryl alcohol and manganese (IV) oxide) to the culture medium of *P. chrysosporium* for the production of lignolytic enzymes increased the decomposition rate of the dye Poly R-478 (Couto et al. 2000). In many cases, it is preferable that suitable organisms excrete the active enzymes into the medium, otherwise transport into the cells becomes limiting for bio-elimination. Processes using immobilized growing cells or immobilized enzymes seem to be more promising than those involving free cells, since immobilization allows repeated use of the microbial cells.

Some disadvantages of biological treatments are the requirement for a large land area, the sensitivity toward diurnal variation, the toxicity of some chemicals, and less flexibility in design and operation.

4.5.6.1 Decolourisation by Mixed Cultures

Utilization of microorganism consortia offers considerable advantages over the use of pure cultures in the degradation of synthetic dyes. Furthermore, mixed culture studies may be more comparable to practical situations. With the increasing complexity of a xenobiotic, one cannot expect to find complete catabolic pathways in a single organism; a higher degree of biodegradation and even mineralization might be accomplished when co-metabolic activities within a microbial community complement one another (Nigam et al. 1996; Khadijah et al. 2009). Using mixed cultures instead of pure cultures, higher degrees of biodegradation and mineralization can be achieved due to synergistic metabolic activities of the microbial community (Ramalho et al. 2004; Khehra et al. 2005; Ali 2010). The individual strains can attack dye molecules at different positions, yielding metabolic end products that may be toxic; these can be further metabolised as nutrient sources to carbon dioxide, ammonia and water by another strain. Other species present may not be involved in bioremediation at all, but can stabilise the overall ecosystem (Kandelbauer and Gübitz 2005). This type of mineralization is the safest way to assure that no potentially harmful and unrecognized intermediate degradation products are released into the environment. Mixed consortia usually do not require sterile conditions and have greater stability towards changes in the prevailing conditions (pH, temperature and feed composition) compared with pure cultures (Ramalho et al. 2004). Therefore, the use of mixed cultures is a good strategy for bioreactors.

Biological activated sludge is the most common type of treatment system using mixed cultures. However, activated sludge technology has several inherent disadvantages, such as low biomass concentration and easy washout. It should be stressed that the composition of mixed cultures can change during the decomposition process due to the metabolism, which can interfere with the control of technologies using mixed cultures.

4.5.6.2 Decolourisation by Isolated Organisms and Their Enzymes

The use of an isolated culture system ensures that the data are reproducible and more readily interpreted. The detailed mechanisms of biodegradation can be understood in terms of biochemistry and molecular biology, and these disciplines can also be used to upregulate enzyme system to generate modified strains with enhanced biodegradation activities. The quantitative analysis of the kinetics of azo-dye decolourisation by a particular bacterial culture can be meaningfully valuable asset. Also, the response of the system to changes in operational parameters can be examined. Many strains and enzymes that can degrade dyes or other pollutants now been commercialized. Enzymes presently under investigation are still expensive

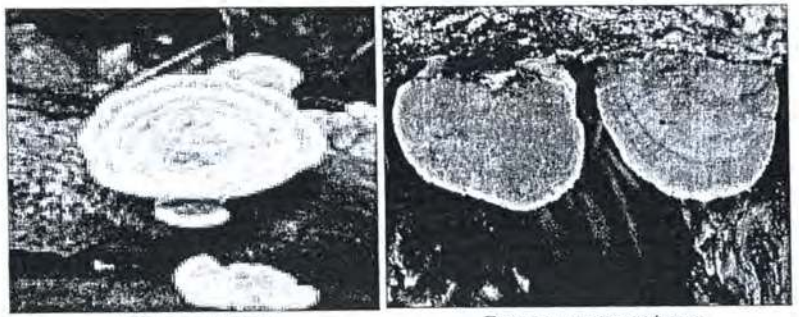

Fig. 4.9 Examples of white-rot-fungi: *Trametes hirsuta* and *Pycnoporus sanguineous*

due to the high cost of isolation, purification and production. Some commercial fungal enzymes and their costs are listed in table 2 of the review by Durán and Esposito (2000).

Filamentous Fungi

By far the most efficient single class of microorganisms in breaking down synthetic dyes is the white-rot fungi (WRF; Fig. 4.9). White-rot fungi are a class of microorganisms that produce non-specific extracellular ligninolytic enzymes capable of extensive aerobic depolymerization and mineralization of lignin (Couto 2009). Lignin is a complex chemical compound abundant in wood, being an integral part of the cell walls of plants. Its degradation is a rate-limiting step in carbon recycling (Ohkuma et al. 2001). Microbial ability to metabolize lignin and its components is one of the plausible evolutionary origins of the degrading pathway of aromatic xenobiotics and/or environmental pollutants. The main extracellular enzymes participating in lignin degradation are lignin peroxidase (ligninase, LiP, EC 1.11.1.14), manganese peroxidase (MnP, EC 1.11.1.13), and the Cu-containing laccase (benzenediol:oxygen oxidoreductase, EC 1.10.3.2). A new group of ligninolytic heme-containing peroxidases, combining structural and functional properties of the LiPs and MnPs, are the versatile peroxidases (VPs). In addition, enzymes involved in hydrogen peroxide production such as glyoxal oxidase (GLOX) and aryl alcohol oxidase (AAO) (EC 1.1.3.7) can be considered to belong to the ligninolytic system (Wesenberg et al. 2003). It is the non-specificity of these enzymes synthesized by WRF that makes them efficient degraders of a wide range of xenobiotics under aerobic conditions, including dyes (Ohkuma et al. 2001; Wesenberg et al. 2003; Couto 2009). *Phanerochaete chrysosporium* was the first species identified that could degrade polymeric synthetic dyes (Glenn and Gold 1983). In addition to enzymatic biodegradation, fungi may decolourise solutions by biosorption (Fu and

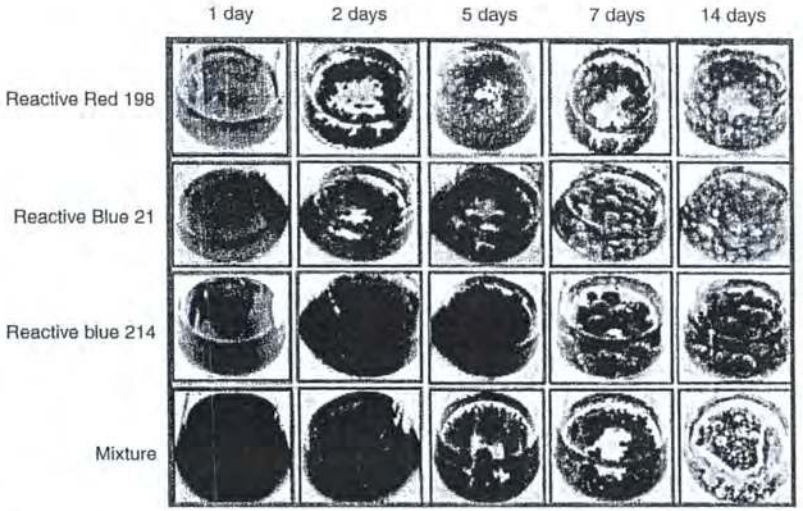

Fig. 4.10 Visual observation of different reactive dyes and a mixture decolourisation by *P. simplicissimum* at increasing incubation times (adapted from Bergsten-Torralba et al. 2009)

Viraraghavan 2002; Zeroual et al. 2006; Prigione 2008a, b). The cell-adsorbed dye can be further enzymatically decolourised.

Long growth cycles and moderate decolourization rates limit the performance of fungal systems (Banat et al. 1996). Fungi growth takes normally from three to seven days. They can be cultivate in different culture media, such as yeast extract, malt extract, agar, starch and potato dextrose using low pHs normally ranging from 3 to 5 (Fu and Viraraghavan 2001). The optimal growth temperature depends on the fungi, but is in the range of 20–35°C. In general, better decolourisation and biodegradation activities are also obtained at acidic or neural pH, and at temperatures between 20 and 35°C. The process can take hours, but more often several days (Ali 2010). For many recent publications on fungi decolourisation of synthetic dyes, see table 2 in Ali (2010). Figure 4.10, is an example of decolourisation by the fungus, *P. Simplicissimum,* of several different single reactive dyes and their mixtures (Bergsten-Torralba et al. 2009). Various type of reactor systems using fungi have been described (Fu and Viraraghavan 2001; Kapdan and Kargi 2002), although, processes using immobilized growing cells look more promising than those using free cells, since the former can be repeatedly and continuously used in the remediation process (Zhang et al. 1999). Furthermore, immobilized cultures are more resilient to environmental perturbations, e.g. pH or exposure to toxic chemicals concentrations, than suspension cultures (Kuhad et al. 2004; Couto 2009). Two interesting reviews on the use of immobilized fungi for dye decolourisation come from Couto (2009) and Mazmanci (2010).

Bacteria

Efforts to isolate bacterial cultures capable of degrading azo dyes started in the 1970s with reports of *Bacillus subtilis* (Horistsu et al. 1977), followed by *Aeromonas hydrophilia* (Idaka and Ogewa 1978) and *Bacillus cereus* (Wuhrmann et al. 1980). Numerous other bacteria capable of dye decolourisation have been found in the interim (Banat et al. 1996; Dave and Dave 2009). Khadijah et al. (2009) isolated 1540 bacteria and screened them for the ability to degrade azo dyes; from the initial screening in microtitre plates, 220 isolates showed decolourisation potential, of which 37 showed higher decolourised zones on dye-incorporated agar plates. In the final screening in liquid medium, 9 proved capable of degrading a wide spectrum of dyes. Bacteria degrade azo dyes reductively under anaerobic conditions to give colourless aromatic amines. These in turn need to be further degraded due to their possible toxic, mutagenic and/or carcinogenic character in humans and animals (Chen 2006). Human intestinal bacteria can also degrade azo dyes to carcinogenic aromatic amines (Chen 2006), which presents a public health problem where low amounts of dyes might be ingested. Anthraquinonic dyes are less susceptible to anaerobic reduction. Whole-cell biodegradation is often carried out by a number of enzymes working sequentially; however, as with other microorganism, only a few of the expressed bacterial enzymes are directly involved in dye biotransformation. The bacterial enzymes involved in the reductive azo bound cleavage are usually azoreductases, whose actions may depend on the presence of other substances such as cofactors, co-substrates or mediators. To avoid the formation of carcinogenic amines, aerobic conditions are preferable in aromatic amine degradation, but it should also be noted that some of them may be auto-oxidized to polymeric structures in the presence of oxygen (Kudlich et al. 1999). Undeniably, the isolation of bacteria capable of aerobic decolourisation and mineralization of dyes has attracted interest, although, especially for sulfonated azo dyes, things have proven difficult (McMullan et al. 2001). Contrary to the unspecific mechanism of azo dye bacterial reduction under anaerobic conditions, aerobic bacteria usually need to be specifically adapted to achieve a significant reductive process. This adaptation involves long-term aerobic growth in continuous culture in the presence of a very simple azo compound. Induction leads to the bacteria synthesis of azoreductases, specific for the reduction of the inducer azo compound, or even others related compounds, in the presence of oxygen (Stolz 2001). Some strains of aerobic bacteria can degrade azo groups by special oxygen-tolerant azo reductases, but they have limited substrate range (Zimmermann et al. 1982; Nachiyar and Rajakumar 2005; Chen 2006). The concepts of anaerobic and aerobic biodegradation will be described in Sects. 4.5.5.4 and 4.5.5.5.

The use of bacteria is influenced by factors at the level of the cell, which in turn will influence the permeability and diffusion of dye molecules. Parameters, such as cell density, enzymes per cell, enzymatic catalytic efficiency, substrate charge and even cell permeability, can be modeled in order to achieve the highest removal rate (Martinez et al. 1999). Generally, unlike fungi, bacteria show better decolourisation and biodegradation activities at basic pH. In comparison to fungi, bacterial decolourisation tends to be faster (Kalyani et al. 2009).

For dye degradation in a bioreactor, immobilization of bacterial cells is also preferable. The advantages of using intact or immobilized cells for biocatalysis is that there is no need to recover and purify the enzymes involved in the process; in addition enzymes encapsulated in the cells may be more resistant to the operating conditions in the long term, in particular for decolourising model or real wastewaters that are rich in salts, additives, surfactants, detergents and others compounds. In addition, costs associated with enzyme purification are negated. Cell immobilization is an effective way to maintain continuous substrate degradation with concomitant cell growth for the treatment of toxic materials. Compared with suspension cells, the main advantage of immobilized cells includes the retention of microorganisms in the reactor and hence protection of cells against toxic substances. The biocatalysts (cells) can be used in repeated cycles, which is of great importance when applied on an industrial scale. Indeed, Advanced Immobilized Cell Reactor technology has been developed specifically to attend for a cost effective treatment system that would accommodate shock load applications and be extremely flexible in its operation. One disadvantage of immobilization is the increased resistance of substrates and products to diffusion through matrices used for immobilization. Owing to the low solubility of oxygen in water and the high local cell density, oxygen transfer often becomes the rate-limiting factor in the performance of aerobic immobilized cell systems. Thus, when aerobic cells are used, aeration technique becomes a very important consideration in bioreactor design technology. Immobilization commonly is accomplished using natural high molecular hydrophilic polymeric gel, such as calcium or sodium alginate, carrageenan and agarose (Palmieri et al. 2005) or many synthetic polymers, such as poly-acrylamide (PAM), polyvinyl alcohol (PVA) (Zhou et al. 2008). Activated carbon has also been used, especially in high performance bioreactors (Walker and Weatherley 1999; van der Zee and Villaverde 2005). The recently developed BIOCOL process (Conlon and Khraisheh 2002) is a commercially available option for the treatment of azo dyes and uses a bacterium isolated from soil contaminated with textile wastewater as the biocatalyst. In this process, the bacterial cells are grown and immobilised on an activated carbon support material that adsorbs the target dye molecules and the potentially toxic amine breakdown products for biodegradation. As well as acting as an absorbent, the activated carbon can potentially promote the degradative activity of the biocatalyst (van der Zee et al. 2001)

Yeast

Very little work has been devoted to the study of the decolourising ability of yeast, most often mentioning sorption as the main cause (Meehan et al. 2000; Donmez 2002). Nevertheless, there are some reports on biodegradation by yeast strains, such as *Candida zeylanoides* (Martins et al. 1999), *Candida zeylanoides* and *Issatchenkia occidentalis* (Ramalho et al. 2002 and 2004, respectively). Yeast cells, like bacteria,

are capable of azo dye reduction to the corresponding amines. Testing adapted and unadapted cultures, Ramalho et al. (2004) found that the azo dye reduction activity was due to a constitutive enzyme and that activities were dependent on intact, active cells. Moreover, they noted that *I. occidentalis* (a dye reducer strain) has an absolute requirement of oxygen. Compared to bacteria and filamentous fungi, yeasts have some of the advantages of both: they not only grow rapidly like bacteria, but like filamentous fungi, they also have the ability to resist unfavourable environments (Yu and Wen 2005). In yeast, the ferric reductase system participates in the extracellular reduction of dyes (Ramalho et al. 2005; Chen 2006). In general, yeasts show better decolourisation and biodegradation activities at acidic or neutral pH.

4.5.6.3 Decolourisation by Isolated Enzymes

Independent of the organism to which they belong, fungi, bacteria or yeast, the degradation of some compounds is catalysed by specific enzymes. Indeed, there is a growing recognition that enzymes can be used in many remediation treatments to target specific pollutants. In this direction, recent biotechnological advances have allowed the production of cheaper and more readily available enzymes through better isolation and purification procedures. At the same time, fundamental studies on enzyme structures and enzymatic mechanisms have been conducted.

The main enzymes involved in dye degradation are the lignin-modifying extracellular enzymes (laccase, lignin peroxidase, phenol oxidase, Mn-dependent peroxidase and Mn-independent peroxidase) secreted by WRF, and the bacterial azoreductases. Because of their high biodegradation capacity, they are of considerable biotechnological interest and their application in the decolourisation process of wastewaters has been extensively investigated (Young and Yu 1997). Several enzymes have been isolated and characterised (Grigorious et al. 2000); however, azo reductases have little activity in vivo (Rus et al. 2000). From an environmental point of view, the use of enzymes instead of chemicals or microorganisms presents several advantages, including the potential for scale-up, with enhanced stability and/or activity, and at a lower cost through using recombinant-DNA technology. Another advantage of using pure enzymes instead of the microorganism is that the expression of enzymes involved in dye degradation is not constant with time, but dependent on the growth phase of the organisms and is influenced by inhibitors that may be present in the effluent. Synthetic or natural redox mediators have, to be added many times to the enzymatic bath in order to achieve the total capacity of the enzyme(s) or even to make their work possible (see Sect. 4.5.6.4).

The major drawback of using enzyme preparations is that once the enzymes become inactivated, it is of no use. Because enzymes can be inactivated by the presence of the other chemicals, it is likely that enzymatic treatment will be most effective in streams that have the highest concentrations of target contaminants but the lowest concentration of other compounds that could interfere with their action. In order to increase the potential use of enzymes in a wastewater bioremediation

process, their immobilisation is recommended for biochemical stability and reuse, thereby reducing the cost (Durán and Esposito 2000; Kandelbauer et al. 2004).

4.5.6.4 Anaerobic Dye Decolourisation

Anaerobic processes convert the organic contaminants principally into methane and carbon dioxide. They usually occupy less space, can treat wastes containing up to 30 000 mg L^{-1} of COD, have lower running costs, and produce less sludge.

Azo dye degradation occurs preferentially under anaerobic or oxygen limited concentrations, acting as final electron acceptors during microbial respiration. Oxygen, when it is present, may compete with the dyes. In many cases the decolourisation of reactive azo dyes under anaerobic conditions is a co-metabolic reaction (Stolz 2001). Several mechanisms have been proposed for the decolourisation of azo dyes under anaerobic conditions (Rus et al. 2000). One of these is the reductive cleavage of the azo bond by unspecific cytoplasmic azo reductases using flavoproteins ($FMNH_2$ and $FADH_2$) as cofactors. A second proposed mechanism is an intracellular, non-enzymatic reaction consisting of a simple chemical reduction of the azo bond by reduced flavin nucleotides. These reductive cleavages, with the transfer of 4 electrons and the respective aromatic amine formation (Fig. 4.11), usually occur with low specific activities but are extremely nonspecific with regard to the organism involved and the dyes converted. Transport of the reduction equivalents from the cellular system to the azo compounds is also important, because the most relevant azo compounds are either too polar and/or too large to pass through the cell membrane (Rau and Stolz 2003). Mediators generally enable or accelerate the electron transfer of reducing equivalents from a cell membrane of a bacterium to the terminal electron acceptor, the azo dye (Kudlich et al. 1997; Rus et al. 2000; Keck et al. 2002; Van der Zee et al. 2001). Such compounds can either result from the aerobic metabolism of certain substances by bacteria themselves or be added to the medium. They are enzymatically reduced by the cells and these reduced mediator compounds in turn reduce the azo group in a purely chemical reaction (Stolz 2001). It has been suggested that quinoide redox mediators with standard redox potentials (E0') between –320 and –50 mV could function as effective redox mediators in the microbial reduction of azo dyes. The first example of an anaerobic cleavage of azo dyes by redox mediators which are naturally formed during the aerobic metabolism of xenobiotic compound was reported by Keck et al. (1997). Dos Santos et al. (2005) studied the impact of different redox mediators on colour removal of

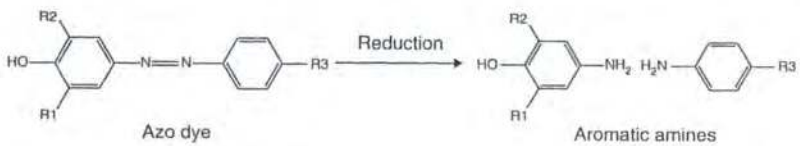

Fig. 4.11 Mechanism of an azo dye reduction

azo dye model compound; up to an eightfold increase in decolourisation rates were achieved compared with mediator-free incubations. Many authors have reported increase of azo dye decolourisation rates in the presence of such molecules, either by membrane-bound respiratory chain or cytosolic enzymes (Kudlich et al. 1997; Rau et al. 2002; 2003; Ramalho et al. 2004; Pearce et al. 2006). The need in some cases for external addition of chemical mediators may increase the cost of the process. In crude extracts and crude enzyme preparations, low molecular weight compounds may be naturally present that act as natural enhancing compounds. The physiology of the possible reactions that result in a reductive cleavage of azo compounds under anaerobic conditions differs significantly from the situation in the presence of oxygen because the redox active compounds rapidly react either with oxygen or azo dyes (Stolz 2001). Therefore, under aerobic conditions, oxygen and dyes compete for the reduced electron carriers. In bioremediation, an anaerobic treatment or pretreatment step can be a cheap alternative compared with an aerobic system because expensive aeration is omitted and the problem with bulking sludge is avoided.

In addition to azo dyes, other classes of dyes have been degraded under anaerobic conditions (McMullan et al. 2001), but the mechanisms are less well described.

4.5.6.5 Aerobic Dye Decolourisation

In aerobic pathways, azo dyes are oxidized without the cleavage of the azo bond through a highly non-specific free radical mechanism, forming phenolic type compounds (Fig. 4.12). This mechanism avoids the formation of the toxic aromatic amines arising under reductive conditions (Chivukula and Renganahathan 1995; Chen 2006; Pereira et al. 2009a).

The main organisms involved in the oxidative degradation of dyes are the fungi WRF by the so-called lignolitic enzymes (Sect. 5.5.2.1). The application of these organisms or their enzymes in dye wastewater bioremediation have attracted increasing scientific attention because they are able to degrade a wide range of organic pollutants, including various azo, heterocyclic and polymerise dyes (Abadulla et al. 2000; Wesenberg et al. 2003). It is also noteworthy that they require mild conditions, with better activities and stability in acidic media and at temperatures from 20 to 35°C. Oxidases can also be found in some bacteria, plants and animals.

Peroxidases, including LiP and MnP, use hydrogen peroxide to promote the one-electron oxidation mechanism of chemicals to free radicals (Figs. 4.13 and 4.14, respectively). Those enzymes have an important role on the cellular detoxification

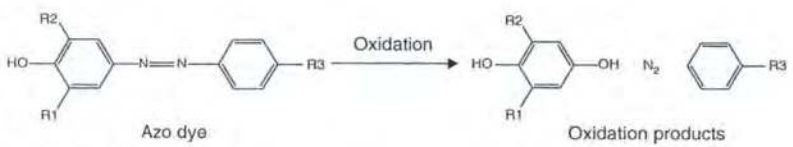

Fig. 4.12 Mechanism of an azo dye oxidation

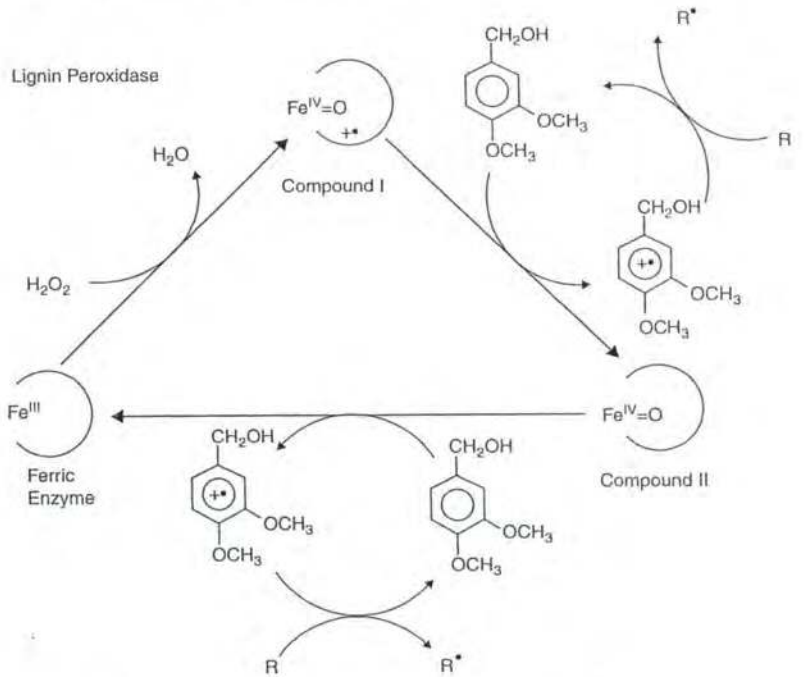

Fig. 4.13 Catalytic cycle of a manganese peroxidase (Cameron et al. 2000)

of organisms by eliminating hydrogen peroxide. They are hemoproteins belonging to the oxidoreductase enzymes that catalyze the oxidation of phenols, biphenols, anilines, benzidines and related hetereoaromatic compounds, for which H_2O_2 is the final electron acceptor (Durán and Esposito 2000). The dye does not need to bind to the enzyme; instead oxidation occurs through simple electron transfer, either directly or through the action of low molecular weight redox mediators (Eggert et al. 1996). Lignin peroxidase was discovered earlier than MnP and exhibits the common peroxidase catalytic cycle. It interacts with its substrates via a ping-pong mechanism, i.e. firstly it is oxidized by H_2O_2 through the removal of two electrons that give compound I, and further oxidized through the removal of one electron to give compound II, which oxidizes its substrate, returning to the resting enzyme. The active intermediates of LiP (i.e. componds I and II) have considerably higher reduction potentials than the intermediates of other peroxidises, extending the number of chemicals that can be oxidised. MnP mechanism differs from that of LiP in using Mn^{+2} as a mediator. Once Mn^{+2} has been oxidized by the enzyme, Mn^{+3} can oxidize organic substrate molecules. MnP compounds I and II can oxidize Mn^{+2} to Mn^{+3}, but compound I can also oxidize some phenolic substrates.

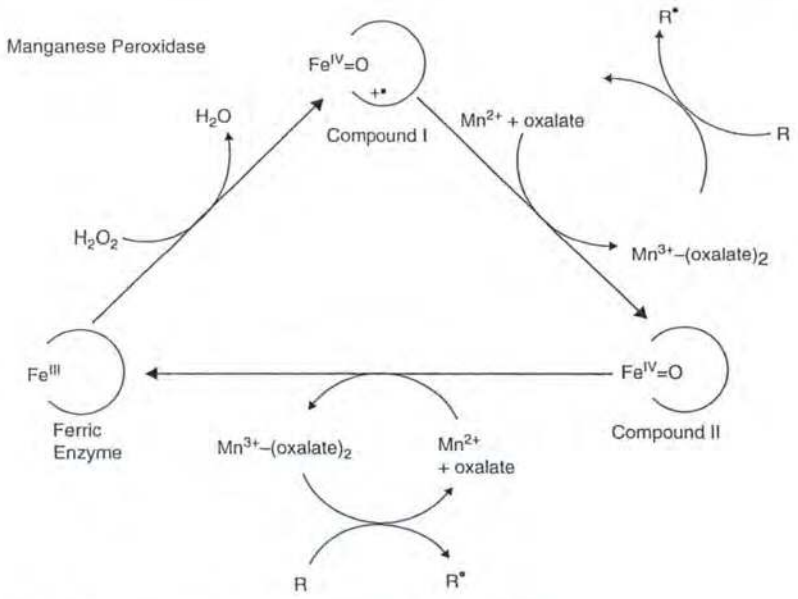

Fig. 4.14 Catalytic cycle of a lignin peroxidase (Cameron et al. 2000)

The use of pure peroxidases for dye degradation, in place of the organism, requires the addition of hydrogen peroxide. The pathways for the degradation of two sulfonated azo dyes by the peroxidise and ligninase of *Phanerochaete chrysosporium* and by the peroxidise of *Streptomyces chromofuscust* have been proposed by Goszczynski et al. (1994). More recently, López et al. (2004) have proposed a degradation pathway of Orange II by MnP enzyme.

Laccases are another group of oxidoreductases, but these use oxygen as the final electron acceptor. This may be advantageous when using the pure enzyme, relatively to peroxidases, since H_2O_2 and cofactors are not needed. Laccases belong to the small group of named multicopper blue oxidase enzymes, and the mechanism of oxidative reactions catalysed by them involves the transfer of 4 single electrons from the substrate to the final acceptor. Their catalytic centres consist of 3 structurally and functionally distinct copper centres (Solomon et al. 1996; Stoj and Kosman et al. 2005). T1 copper ("blue copper") is a mononuclear centre involved in the substrate oxidation, whereas T2 and T3 form a trinuclear centre involved in the reduction of oxygen to water (Fig. 4.15). Owing to their high relative nonspecific oxidation capacity, laccases have proven useful for diverse biotechnological applications including degradation of dyes (Abadulla et al. 2000; Husain 2006) and organic synthesis (Eggert et al. 1996; Riva 2006; Schroeder et al. 2007). The first described laccase had plant origin, the Japanese lacquer tree, *Rhus vernicefera*

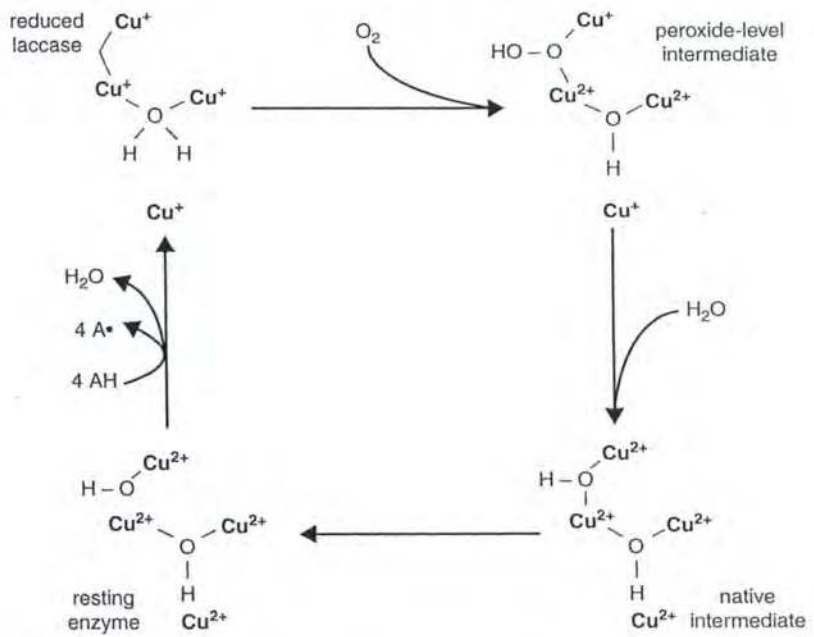

Fig. 4.15 Catalytic cycle of a laccase (Wesenberg et al. 2003)

(Yoshida and Takemori 1997). Nowadays, most of the known laccases have fungal (e.g. white-rot fungi) or plant origins, although a few have recently been identified and isolated from bacteria (Claus 2003; Gianfreda et al. 1999). The ability of a bacterial laccase (CotA) to degrade a wide range of azo and anthraquinonic dyes was reported for the first time by Pereira et al. (2009a, b). The mechanism of azo dye degradation has been described by some authors (Chivukula and Renganahathan 1995: Zille et al. 2005; Pereira et al. 2009a). Antraquinonic dyes are also an important class of dyes, although there is limited information on their physicochemical or biological degradation, and even less on the molecular mechanisms of transformation (Pereira et al. 2009b). Based on the characterisation of intermediates and final products of the reaction, Pereira et al. (2009b) described the mechanistic pathway for the biotransformation of the anthraquinonic dye AB62 by the bacterial CotA-laccase.

The mechanism of Indigo dye (the most important dye in the manufacturing of blue jeans) degradation by a laccase has also been described (Campos et al. 2001). The dye is cleaved under laccase catalyzed electron transfer to give isatin, which, after further decarboxylation, is catalyzed to the final stable oxidation product, anthranilic acid (Fig. 4.16). Likewise, in peroxidise-catalyzed decolourisation of Indi-

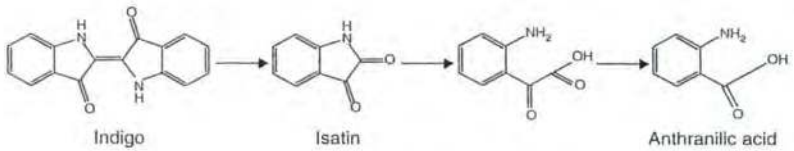

Fig. 4.16 Mechanism of Indigo oxidation by purified laccases from *Trametes hirsuta* and *Sclerotium rolfsii* (Campos et al. 2001)

go Carmine, isatin sulfonic acid is formed, although a stable red oxidation product, probably a dimeric condensation product, was obtained (Podgornik et al. 2001).

4.5.6.6 Combination of Anaerobic/Aerobic Processes for Dye Treatment

Many dyes used in textile industry cannot be degraded aerobically as the enzymes involved are dye-specific. Anaerobic reduction of azo dyes is generally more satisfactory than aerobic degradation, but the intermediate products (carcinogenic aromatic amines) must be further degraded. These colourless amines are, however, very resistant to further degradation under anaerobic conditions, and therefore aerobic conditions are required for complete mineralisation (Melgoza et al. 2004; Forgacs et al. 2004; Pandey et al. 2007). For the most effective wastewater treatment, 2-stage biological wastewater treatment systems, are then necessary in which an aerobic treatment is introduced after the initial anaerobic reduction of the azo bond (Sponza and Işik 2005; Van der Zee and Villaverde 2005; see Fig. 4.17). The balance between the anaerobic and aerobic stages in this treatment system must be carefully controlled because it may become darker during re-aeration of a reduced dye solution. This is to be expected, since aromatic amines are spontaneously unstable in the presence of oxygen. Oxidation of the hydroxyl and amino groups to quinines and quinine imines can occur and these products can also undergo dimerisation or polymerisation, leading to the development of new darkly coloured chromophores (Pereira et al. 2009a, b). However, with the establishment of correct operating conditions, many strains of bacteria are capable of achieving high levels of decolourisation when used in a sequential anaerobic/aerobic treatment process (Steffan et al. 2005; van der Zee and Villaverde 2005). Aerobic biodegradation of many aromatic amines has been extensively studied (Brown and Laboureur 1983; Pinheiro et al. 2004; van der Zee and Villaverde 2005), but these findings may not apply to all aromatic amines. Specially sulfonated aromatic amines are often difficult to degrade (Razo-Flores et al. 1996; Tan and Field et al. 2000; Tan et al. 2005). Aromatic amines are commonly not degraded under anaerobic conditions. Melgoza et al. (2004) studied the fate of Disperse Blue 79 in a two-stage anaerobic/aerobic process; the azo dye was biotransformed to amines in the anaerobic stage and an increase of toxicity was obtained; the toxic amines were subsequently mineralized in the aerobic phase, resulting in the detoxification of the effluent.

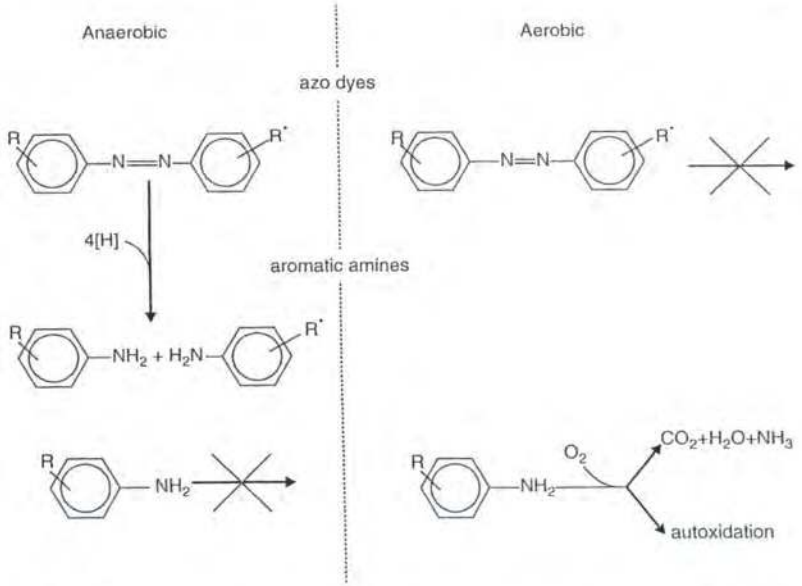

Fig. 4.17 Azo dye degradation in a two-step (aerobic/anaerobic) process (van der Zee and Villaverde 2005)

The 2-stage treatment systems can be the result of many different combinations, such as 2 biological or 2 physicochemical processes, but also of combinations of both these types. The choice for the best treatments is related to many other factors, such as the type of effluent, the availability of the methods, the locality where it is to be applied, and others.

4.5.6.7 Factors Affecting Biodegradation of Dyes

Ecosystems are dynamic environments with variable abiotic conditions, e.g. pH, temperature, dissolved oxygen, nitrate concentration, metals, salts, etc. Microorganisms are affected by changes in these parameters, and consequently their decomposing activities are also affected. Other non-dye related parameters are the type and source of reduction equivalents, bacteria consortium, and cell permeability. Enzymes synthesised by microorganisms, when isolated, are also sensitive to the medium culture conditions in which they are applied. Usually, they proceed better in environments more suitable for the original microorganism. Textile wastewaters result from different classes of dyes, and consequently vary in their composition and pH. It is therefore important, while evaluating the potential of different microorganisms for dye degradation, to consider the effects of other present com-

pounds. Optimization of abiotic conditions will greatly help in the development of industrial-scale bioreactors for bioremediation. Optimal pH and temperatures are always related to the environment where the organisms were collected, but usually fungi work better, either for growth or compounds degradation, in acidic or neutral media, whereas bacteria prefer alkaline conditions. Dye decolourisation proceeds better under acidic conditions for fungi and fungal enzymes (Abadulla et al. 2000; Kandelbauer et al. 2004; Almansa et al. 2004; Zille et al. 2005), but under alkaline conditions for bacteria and bacterial enzymes (Pereira et al. 2009a). The optimal temperatures of microorganisms usually range from 20 to 35°C, but there are also others that tolerate higher values, although the stability may be compromised. Beyond the optimum temperature, the degradation activities of the microorganisms decrease because of slower growth, reproduction rate and the deactivation of enzymes responsible for degradation.

Biodegradation of azo dyes and textile effluents can be affected by dye related parameters, such as class and type of azo dye, reduction metabolites, dye concentration, dye side-groups and organic dye additives. Microbial activity can decrease with increasing dye concentration, which can be attributed to the toxicity of the dyes to the growing microbial cells at higher concentrations (in the biodegradation) and/or cell saturation (biosorption). In the most enzymatic decolourisation studies, the kinetics are described by Michaelis-Menten model and an increase of the rate with increase the dye concentration is observed up to a certain concentration (saturation). At dye amounts higher than the optimal, the rates usually remain constant due to saturation, but there are also some cases of inhibition at concentrations higher than the optimal. The inhibition concentration of dye is not the same for all the microorganisms, and for the same organisms, the inhibition concentration will depend on the dye. Adaptation of a microbial community to the compound is very useful in improving the rate of decolourisation process, due to the natural expression of genes encoding for enzymes responsible for its degradation, when previously exposed (Ramalho et al. 2004). The fact that some dyes are biodegraded and others not, even under the same conditions, is explained by the role of the chemical structure of the dye on the process. Even when belonging to the same class and type, dyes differ in their structure and present different p_{Ka} and potential redox. Zille et al. (2004) studied the biodegradation under aerobic conditions of azo dyes by yeasts with reducing activity and by an oxidative enzyme, laccase, with or without mediator; they compared these 2 approaches on the basis of the electrochemical properties of dyes and bioagents. A linear increase of dye decolourisation with decreasing redox potential of dye was obtained with laccase and laccase/mediator systems; in the reductive approach, they observed that the less negative the redox potential of the azo dye, the more favourable (and faster) its reduction. The redox potential should reportedly be below –450 to –500 mV for azo dye reduction to occur (Delée et al. 1998). It is worth mentioning that the redox potential is influenced by other external factors, such as the pH of the solution. The redox potential of the enzymes may also be involved, although in many cases, enzymes with redox potential lower than that of the dyes can decolourise them. These facts can be understood in the light of the Nernst equation; any redox reaction is dependent on the formal redox potential, and on the concentrations of the reduced and oxidized species (Zille et al. 2004).

Little information is available in the literature describing quantitatively the effects of dye chemical structure on the reactivity towards laccase oxidation. Almansa et al. (2004) have synthesized 22 model azo-dyes of chemically very similar structure and studied the effect of substituents on the enzymatic kinetics of their decolourisation. The model dyes only differed in nature and position of the substituent on the phenyl ring, which carried either a single methyl ($-CH_3$), trifluoromethyl ($-CF_3$), fluoro (–F), chloro (–Cl), bromo (–Br), nitro ($-NO_2$), or hydroxy (–OH) group in the ortho, meta or para-position with respect to the azo linkage. Without the assistance of an electron mediator, enzymatic degradation took place only with the hydroxy-substituted model azo-dyes. All other dyes were only degraded in the presence of mediator, except those substituted with trifluoromethyl that were not degraded at all. The electron withdrawing effect of 3 fluorine atoms proved strong enough to completely prevent the reaction. In general, they found that electron-withdrawing substitutents diminish reaction rates whereas electron-donating groups enhanced the susceptibility of the dye towards oxidative attack. Similar conclusions were previous reported by Chivukula and Renganathan (1995) and Kandelbauer et al. (2004).

Wastewaters from textile processing and dyestuff manufacture industries contain also substantial amounts of salts in addition to azo dye residues. Thus, microbial species capable of tolerating salt stress will be beneficial for treating such wastewaters. High salt concentrations can also inhibit enzyme activity.

All the referred factors have to be considered and studied for a full-scale application. The influence of the flow rate of the effluent, the nature (concentration) of the effluent, the pursued extent of treatment, the location, the climatic conditions and the configuration of the reactor are all of great importance, not only for the success of the process itself, but also in terms of making it cost-effective.

4.5.6.8 Genetic Engineering of Dye Degrading Organisms

The vast majority of the current publications in the field of the synthetic dyes removal from waters have dealt with the various aspects of the application of microbiological methods and techniques, with the search for new microorganisms providing improved decomposition rates, and with the elucidation of the principal biochemical and biophysical mechanisms underlying the decolourisation process of dyes. Bioprocesses, whether involving the microorganisms themselves or their enzymes, are sufficiently versatile to be customised. Identification, isolation, and transfer of genes encoding degradative enzymes can greatly help in designing microbes with enhanced degradation capabilities. Thus, acclimatization and genetic engineering can both help in designing "super-degraders". Of the two approaches, acclimatization is natural, since in this case the built-in genetic setup of the microorganism is not disturbed; only some components are enabled. On the other hand, in genetic engineering, the natural genetic set-up of a microorganism is changed by incorporating a new gene or genes. Therefore, many scientists—especially environmentalists—are skeptical about the usefulness of genetically modified organisms. They fear that such modified organisms may create new environmental problems

(Ali 2010). However, there is evidence that gene manipulation for the creation of recombinant strains with higher biodegradation capacity will be applied in the future. By cloning and transferring genes encoding for dye degrading enzymes, organisms could be designed that combine the abilities of mixed cultures within a single specie. A number of genes conferring the ability of dye decolourising have been identified. Successful decolourisation of an azo dye using *Escherichia coli* carrying the azoreductase gene from a wild-type *Pseudomonas luteola* has been reported (Chang et al. 2000; Chang and Kuo 2000). This approach could become a useful alternative for shortening the extended time-periods otherwise needed to adapt appropriate cultures and isolated strains, respectively. CotA-laccase, a bacterial enzyme from *Bacillus subtilis* cloned and over-expressed in *E. coli*, has proved to be a thermoactive and intrinsically thermostable enzyme with a high capacity for the decolourisation of azo and anthraquinonic dyes (Pereira et al. 2009a, b). The expression level of CotA-laccases in different *E. coli* host strains, growing under different culture conditions, was compared and a high-throughput screenings for the oxidation of dyes with high potential redox developed by Brissos et al. (2009).

4.6 Products Identification and Mechanisms of Dye Degradation

We have already pointed out that pollutant degradation, including dyes, vary according to non-related and related dye parameters. Identification of the products from synthetic dyes biodegradation is most helpful in determining the mechanistic pathways involved. Such findings are not only important in the knowledge about the fate of organic pollutants, but indirectly in the assessment of the toxicity of the intermediates and main products, and also in describing the microbial system and/or enzymatic activities. Different microorganisms/enzymes may have different pathways of degradation depending on the particular dye structure; thus the strategy of the microbial system for dye degradation and many other factors has to be studied. Dyes, as colourants, absorb in the visible region of the spectra and each one has a maximal wavelength, depending on its visible colour (see Sect. 4.2); therefore, the easiest way to monitor dye degradation is by means of spectrophotometry, following the decrease in its absorbance. By this technique, all the molecules present are quantified, and intermediates and degradation products will contribute to the spectra absorbance. Various basic and advanced instrumental techniques of chromatography such as gas chromatography (GC), high performance liquid chromatography (HPLC), nuclear magnetic resonance spectroscopy (NMR), mass spectrometry (Ion-trap, MALDI) and capillary electrophoresis (CE) are available to assist in the isolation and characterization of the intermediates and products of dye degradation, thereby giving new insight into the mechanism of biodegradation. Prior procedures of extraction of the aqueous sample with an organic solvent or filtration are adopted

when a heterogeneous catalyst or solid reactant is employed, or when a pre-separation is needed.

Quantification of CO_2 and NH_3 produced in incubated culture media can also provide additional information. Recent studies on the on the mechanisms and pathways using those techniques have been published by López et al. (2004), Vanhulle et al. (2008); Bafana et al. (2009) and Pereira et al. (2009a, b).

4.7 Conclusions

The management of textile industrial effluents is a complicated task, taking into consideration the complexity of the waste compounds that may be present, in addition to the dyes, and the numerous established options for treatment and reuse of water. Wide ranges of water pH, temperature, salt concentration and in the chemical structure of numerous dyes in use today add to the complication. Economical removal of colour from effluents remains an important problem, although a number of successful systems employing various physicochemical and biological processes have been successfully implemented. Regulatory agencies are increasingly interested in new, efficient, and improved decolourisation technologies. Solid and evolving scientific knowledge and research is of the utmost relevance for the effective response to current needs. In view of the requirement for a technically and economically satisfactory treatment, a flurry of emerging technologies are being proposed and are at different stages of being tested for commercialization. A broader validation of these new technologies and the integration of different methods in the current treatment schemes will be most likely in the near future, rendering them both efficient and economically viable.

Conventional physicochemical treatments are not always efficient. The high cost, the generation of sludge and of other pollutants, and the need for sophisticated technologies are limiting factors as well. Bioremediation of textile effluents is still seen as an attractive solution due to its reputation as a low-cost, sustainable and publicly acceptable technology. Microorganisms are easy to grow and the use of their isolated enzymes for textile dyes degradation is not expensive in relative terms because there is no need for high purity levels in treating effluents. Many microorganisms and enzymes have been isolated and explored for their ability and capacity to degrade dyes. Others have been modified by the genetic engineering tools to obtain "super and faster degraders". Biological processes may require two stages, especially for azo dyes, in which dyes are reduced in the first anerobic step to their respective aromatic amines, which are then oxidized and mineralized in a final aerobic step. In some cases, a combination of biological with physical—such as adsorption or filtration, or chemical such as coagulation/oxidation—processes may be necessary to achieve the desirable goal. Several low cost and efficient sorbents including natural wastes are very promising, not only due to the lower cost and high availability, but also a new utility is granted to those wastes.

4.8 Future Perspectives

The increasing manufacture and application of synthetic dyes, taking into account their impact in the environment, needs an effective response in terms of modern and viable treatment processes of coloured effluents, prior to their discharge as waste into waterways. Biodegradation of synthetic dyes using different microorganisms and isolated enzymes offer a promising approach by themselves or in combination with conventional treatments.

The complexity of dyes degradation and the existence of an immense variety of structurally different dyes, indicates the need for more research. Moreover, the most of the available studies on dye degradation are referent to azo dyes; the studies shall be extended to the other classes: anthraquinone, indigoid, xanthene, arylmethane and phthalocyanine derivatives. The pathways for dye degradation are also still not totally understood; walk in that direction has high relevance for the development of future modern technology. The increasing research in microbiology, molecular biology, chemistry and genetic fields associated with the degradation technology, are fundamental for that knowledge. Additionally, the effect of coloured substances and their products either in the environment or during the treatment processes will be better evaluated and understood. That better understand, in turn, will leads to a technology improvement and application of more efficient, either from existent or new, treatment processes. New microorganisms and enzymes, with broader substrate specificity and higher activity, will also be found, isolated and studied for their ability and capacity as key agents in pollution remediation. Through the benefits of genetic engineering, random or selective modification of the microorganisms and enzymes can greatly help in designing microbes with higher catalytic power for a wider range of compounds. Optimization of the remediation process in terms of time, efficiency, stabilization and, as consequence, in costs will gain from this. The knowledge and evolution in biochemical, biological and process engineering is also essential for the establishment of processes at industrial scale. Promising processes seem to be the combination of more than one treatment, either biologic or chemical, for the complete mineralization and detoxification of the coloured effluent.

The study and implementation of the new treatments shall not only be focused on pollution reduction, but also in the reuse of water and exploitation of the final by-products for other applications.

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Chapter 5
Molecular Detection of Resistance and Transfer Genes in Environmental Samples

Elisabeth Grohmann and Karsten Arends

Contents

Abstract Horizontal plasmid transfer is the most important means of spreading resistance to antibiotics and heavy metals, as well as virulence genes, to closely and remotely related microorganisms thereby increasing the horizontal gene pool in so diverse habitats such as soils, wastewater, aquifer recharge systems and glacier ice. An overview about the currently used molecular tools to detect and quantify the abundance of antibiotic and heavy metal resistance and transfer genes in aquatic and terrestrial environments is provided. Habitats studied range from nutrient rich environments such as manured agricultural soils to oligotrophic habitats such as drinking water or glaciers in the Antarctic. The state of the art in antibiotic and heavy metal resistance mechanisms and monitoring of conjugative transfer factors

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