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ScienceDirect
Bioresource Technology 98 (2007) 2369–2385
BIORESOURCE TECHNOLOGY

Review

# Review paper on current technologies for decolourisation of textile wastewaters: Perspectives for anaerobic biotechnology

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Received 23 March 2006; received in revised form 7 November 2006; accepted 11 November 2006
Available online 3 January 2007

**Abstract**

Dyes are natural and xenobiotic compounds that make the world more beautiful through coloured substances. However, the release of coloured wastewaters represents a serious environmental problem and a public health concern. Colour removal, especially from textile wastewaters, has been a big challenge over the last decades, and up to now there is no single and economically attractive treatment that can effectively decolourise dyes. In the passed years, notable achievements were made in the use of biotechnological applications to textile wastewaters not only for colour removal but also for the complete mineralization of dyes. Different microorganisms such as aerobic and anaerobic bacteria, fungi and actinomycetes have been found to catalyse dye decolourisation. Moreover, promising results were obtained in accelerating dye decolourisation by adding mediating compounds and/or changing process conditions to high temperatures. This paper provides a critical review on the current technologies available for decolourisation of textile wastewaters and it suggests effective and economically attractive alternatives.


*Keywords:* Azo dyes; Colour removal; Textile wastewaters; Redox mediators; Anaerobic

## 1. Introduction

With the increased demand for textile products, the textile industry and its wastewaters have been increasing proportionally, making it one of the main sources of severe pollution problems worldwide. In particular, the release of coloured effluents into the environment is undesirable, not only because of their colour, but also because many dyes from wastewater and their breakdown products are toxic and/or mutagenic to life (Weisburger, 2002). Without adequate treatment these dyes are stable and can remain in the environment for an extended period of time. For instance, the half-life of hydrolysed Reactive Blue 19 (RB19) is about 46 years at pH 7 and 25 °C (Hao et al., 2000). In addition to the environmental problem, the textile industry consumes large amounts of potable water. In many countries where potable water is scarce, this large water consumption has become intolerable and wastewater recycling has been recommended in order to decrease the water requirements.

Textile wastewaters are characterized by extreme fluctuations in many parameters such as chemical oxygen demand (COD), biochemical oxygen demand (BOD), pH, colour and salinity (Table 1). The wastewater composition will depend on the different organic-based compounds, chemicals and dyes used in the industrial dry and wet-processing steps (Talarposhti et al., 2001; Dos Santos et al., 2006a).

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doi:10.1016/j.biortech.2006.11.013

**Nomenclature**

| | |
|---|---|
| ADMI | American dye manufacturer institute |
| $AH_2QDS$ | anthrahydroquinone-2,6-disulfonate (reduced form of AQDS) |
| AO7 | acid orange 7 |
| AOP | advanced oxidation processes |
| AOX | adsorbable organic halogens |
| AQDS | anthraquinone-2,6-disulfonate |
| AQS | anthraquinone-2-sulfonate |
| AU | absorbance unit |
| BOD | biological oxygen demand |
| COD | chemical oxygen demand |
| CT | carbon tetrachloride |
| $E'_0$ | standard redox potential (pH 7) |
| $E_a$ | activation energy (kJ/mol) |
| EGSB | expanded granular sludge bed |
| EOP | electrochemical oxidation potential |
| FAD | flavin adenine dinucleotide |
| $FADH_2$ | reduced form of FAD |
| FMN | flavin adenine mononucleotide |
| $FMNH_2$ | reduced form of FMN |
| HRT | hydraulic retention time |
| IC-value | value exerting 50% reduction in methanogenic activity |
| NAD | nicotinamide adenine dinucleotide |
| NAD(P) | nicotinamide adenine dinucleotide phosphate |
| NAD(P)H | reduced form of NAD(P) |
| NADH | reduced form of NAD |
| OLR | organic loading rate |
| PVA | polyvinyl alcohol |
| RB19 | reactive blue 19 |
| Riboflavin | vitamin B2 |
| TDS | total dissolved solids |
| TS | total solids |
| UASB | upflow anaerobic sludge bed |
| VFA | volatile fatty acids |
| VSS | volatile suspended solids |
| WL | wavelengths |

The most common textile-processing set-up (Fig. 1) consists of desizing, scouring, bleaching, mercerising and dyeing processes (EPA, 1997; Dos Santos, 2001; Dos Santos et al., 2006a). *Sizing* is the first preparation step, in which sizing agents such as starch, polyvinyl alcohol (PVA) and carboxymethyl cellulose are added to provide strength to the fibres and minimize breakage. *Desizing* is the employed next to remove sizing materials prior to weaving. *Scouring* then removes impurities from the fibres by using alkali solution (commonly sodium hydroxide) to breakdown natural oils, fats, waxes and surfactants, as well as to emulsify and suspend impurities in the scouring bath. *Bleaching* is the step used to remove unwanted colour from the fibres by using chemicals such as sodium hypochlorite and hydrogen peroxide. *Mercerising* is a continuous chemical process used to increase dye-ability, lustre and fibre appearance. In this step a concentrated alkaline solution is applied and an acid solution washes the fibres before the dyeing step. Finally, *Dyeing* is the process of adding colour to the fibres, which normally requires large volumes of water not only in the dyebath, but also during the *rinsing step*. Depending on the dyeing process, many chemicals like metals, salts, surfactants, organic processing assistants, sulphide and formaldehyde, may be added to improve dye adsorption onto the fibres. Fig. 1 shows some potential pollutants from cotton processing operations in which the desizing/scouring and dyebath/rinsing wastewaters are mainly composed of organic pollutants and colour-causing pollutants, respectively (Snowden-Swan, 1995).

## 2. Dye classification and mechanism of fixation

### 2.1. Dye classification

Dyes are classified according to their application and chemical structure. They are composed of a group of atoms responsible for the dye colour, called chromophores, as well as an electron withdrawing or donating substituents that cause or intensify the colour of the chromophores, called auxochromes (Christie, 2001). The most important

Table 1
Characterisation of the cotton wet processing wastewaters (adapted from Correia et al. (1994); Orhon et al. (2001); Mattioli et al. (2002); Bisschops and Spanjers (2003); Dos Santos et al. (2006a))

| Process | COD (g/l) | BOD (g/l) | TS (g/l) | TDS (g/l) | pH | Colour (ADMI) | Water usage (l/kg) |
|---|---|---|---|---|---|---|---|
| Desizing | 4.6–5.9 | 1.7–5.2 | 16.0–32.0 | – | – | – | 3–9 |
| Scouring | 8.0 | 0.1–2.9 | 7.6–17.4 | – | 10–13 | 694 | 26–43 |
| Bleaching | 6.7–13.5 | 0.1–1.7 | 2.3–14.4 | 4.8–19.5 | 8.5–9.6 | 153 | 3–124 |
| Mercerising | 1.6 | 0.05–0.1 | 0.6–1.9 | 4.3–4.6 | 5.5–9.5 | – | 232–308 |
| Dyeing | 1.1–4.6 | 0.01–1.8 | 0.5–14.1 | 0.05[a] | 5–10 | 1450–4750 | 8–300 |

COD, chemical oxygen demand; BOD, biochemical oxygen demand; TS, total solids; TDS, total dissolved solids; ADMI, American dye manufacturer institute.
Obs.: [a]In case of some reactive dyes the salt concentration in the dyebath (dyeing process) can reach concentrations up to 60–100 g/L. Therefore, the values listed in the table can vary enormously depending on the type of the fibre and dye class.

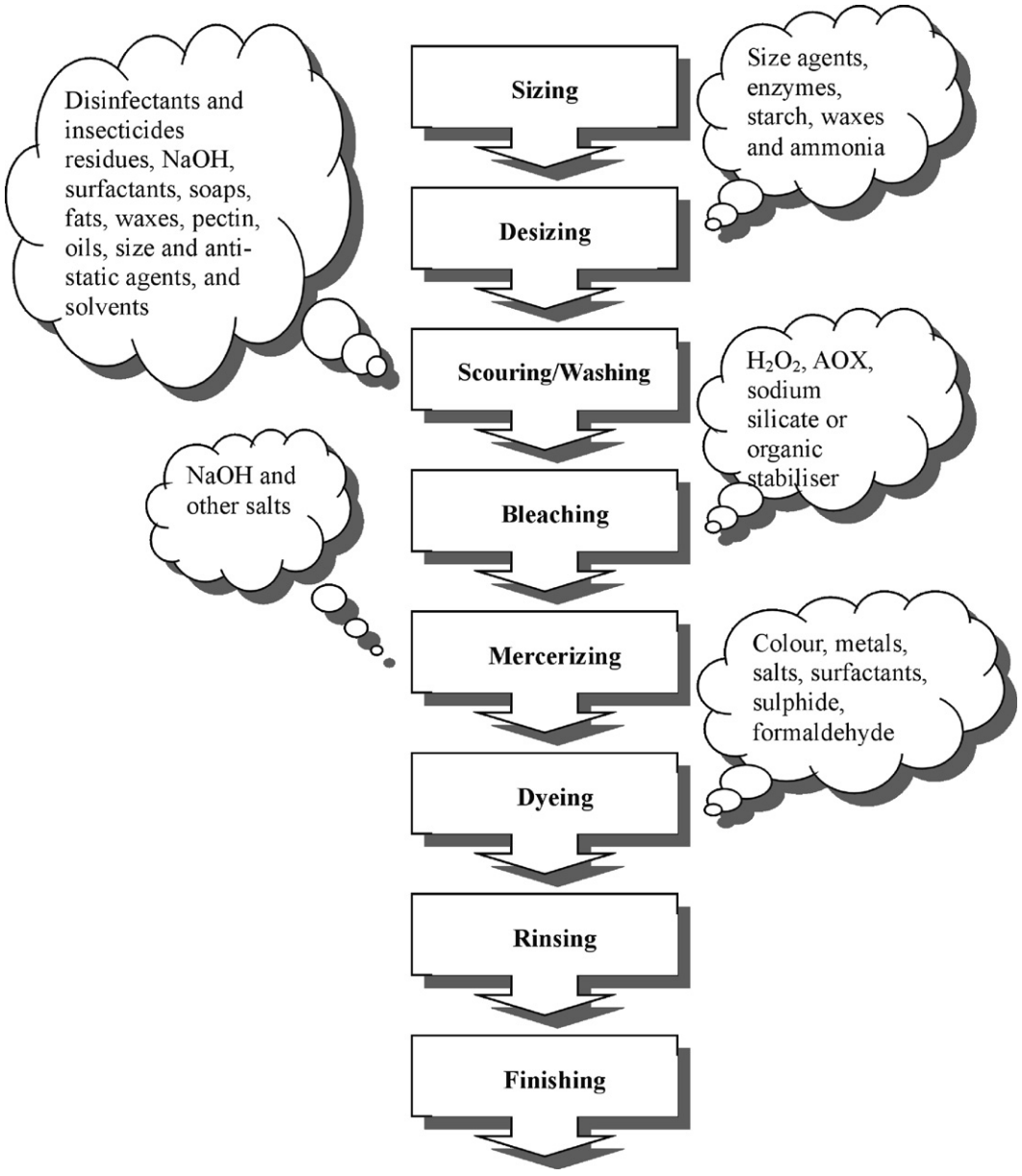


Fig. 1. Schematic of operations involved in textile cotton industry and the main pollutants from each step (adapted from EPA (1997); Mattioli et al. (2002); Dos Santos et al. (2006a)). AOX: Adsorbable Organic Halogens.

chromophores are azo (–N=N–), carbonyl (–C=O), methine (–CH=), nitro ($–NO_2$) and quinoid groups. The most important auxochromes are amine ($–NH_3$), carboxyl (–COOH), sulfonate ($–SO_3H$) and hydroxyl (–OH). It is worth to mention that the sulfonate groups confer very high aqueous solubility to the dyes. The auxochromes can belong to the classes of reactive, acid, direct, basic, mordant, disperse, pigment, vat, anionic and ingrain, sulphur, solvent and disperse dye (Welham, 2000). Fig. 2 gives an illustration of the chemical structure of several dye molecules.

It is estimated that almost $10^9$ kg of dyes are produced annually in the world, of which azo dyes represent about 70% by weight (Zollinger, 1987). This group of dyes is characterised by reactive groups that form covalent bonds with OH–, NH–, or SH– groups in fibres (cotton, wool, silk, nylon). Azo dyes are mostly used for yellow, orange and red colours (Christie, 2001). To obtain the target colour, normally a mixture of red, yellow and blue dyes is applied in the dyebaths. These three dyes do not necessarily have the same chemical structure. They might contain many different chromophores, in which azo, anthraquinone and phthalocyanine dyes are the most important groups (Hao et al., 2000). Anthraquinone dyes constitute the second most important class of textile dyes, after azo dyes (Baughman and Weber, 1994). Anthraquinone dyes have a wide range of colours in almost the whole visible spectrum, but they are most commonly used for violet, blue and green colours (Christie, 2001; Fontenot et al., 2003).

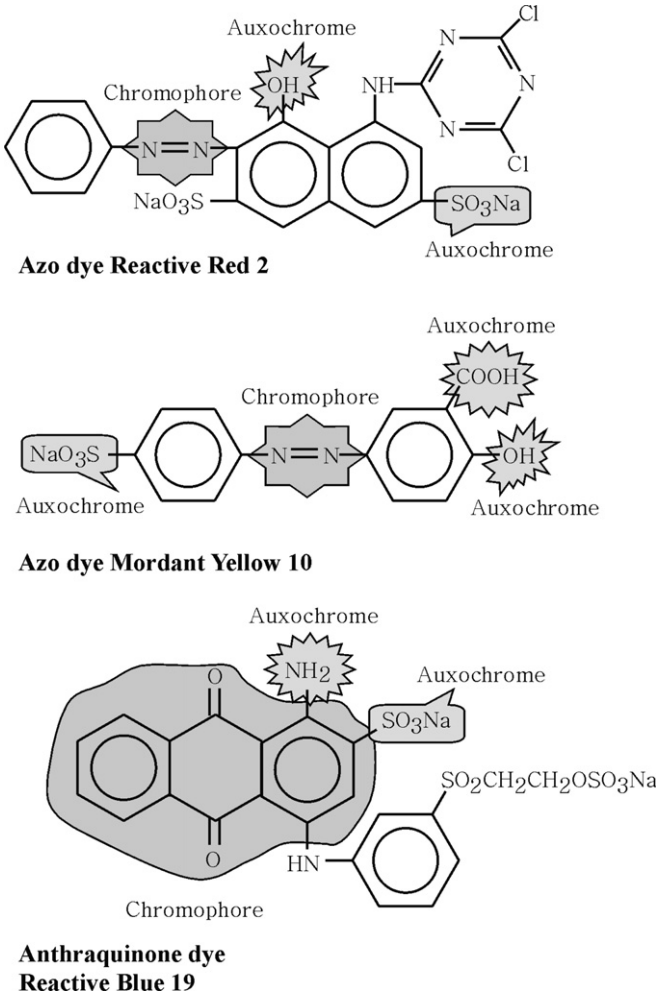


Fig. 2. Examples of dye-auxochromes and–chromophores for azo and anthraquinone dyes.

### 2.2. Fixation mechanisms

Fibres can take up dyes as a result of van der Waals forces, hydrogen bonds and hydrophobic interactions. The uptake of the dye in fibres depends on the dye nature and its chemical constituents. The strongest dye-fibre attachment is a result of a covalent bond with an additional electrostatic interaction where the dye ion and fibre have opposite charges (Welham, 2000).

In alkaline conditions, i.e. pH 9–12 and salt concentration from 40 to 100 g/l, and at high temperatures (30–70 °C), dyes form reactive groups such as vinyl sulfone ($-SO_4-CH{=}CH_2$) and chlorotriazinyl, that form bonds with the fibres. However, the reactive groups undergo hydrolysis, i.e. a spontaneous reaction that occurs in the presence of water, and because the products do not have any affinity with the fibres, they do not form a covalent bond. Therefore, a high amount of dye constituents are discharged in the wastewater (Hao et al., 2000). The fixation efficiency varies with the class of azo dye used, which is around 98% for basic dyes and 50% for reactive dyes (O'Neill et al., 1999). Large amounts of salts such as sodium nitrate, sodium sulphate and sodium chloride are used in the dyebath (Carliell et al., 1998), as well as sodium hydroxide is widely applied to increase the pH to the alkaline range. It is estimated that during the mercerising process the weight of these salts can make up 20% of the fibre weight (EPA, 1997).

## 3. Colour measurement in coloured wastewaters

The electromagnetic spectrum can be divided in three different regions: ultraviolet, visible light and infrared (Table 2). Although visible light is considered to be between the wavelengths of 350 and 780 nm, the human eye can normally detect radiations between the wavelengths of 380 and 720 nm (Christie, 2001).

Colour in water can be either a result of natural phenomena like the presence of humic substances, natural metallic ions, e.g. iron and manganese, and/or plankton; or the colour can also result from an artificial phenomenon like the discharge of dyes and pigments. Industrial processes such as textile, chemical and pharmaceutical industries may discharge large amounts of coloured wastewaters into water bodies. In general, colour in water is classified in terms of true colour, i.e. when the sample is turbidity-free, or apparent colour, i.e. when the sample is measured without previous treatment (APHA, 1998). The most common methods to measure the colour of water and wastewater are visual comparison and spectrophotometry, although there is still a lack of a universal method to classify coloured wastewater emissions. By *visual comparison*, colour is quantified by comparing the sample colour with either known concentrations of coloured standards (normally a platinum–cobalt solution), or properly calibrated colour disks. This method is currently used in water treatment plants as a control parameter mainly because of its simplicity, but it is not applicable for highly coloured industrial wastewaters. In the *spectrophotometric method* (Table 3), colour-measuring protocols differ between the

Table 2
Regions of the electromagnetic spectrum and relationship between wavelength and colour (Christie, 2001)

| Electromagnetic region | Wavelength (nm) | Colour perception |
|---|---|---|
| Ultraviolet | <350 | nd |
| Visible light | 350–400 | nd |
| | 400–435 | Violet |
| | 435–480 | Blue |
| | 480–490 | Greenish-blue |
| | 490–500 | Bluish-green |
| | 500–560 | Green |
| | 560–580 | Yellowish-green |
| | 580–595 | Yellow |
| | 595–605 | Orange |
| | 605–750 | Red |
| | 750–780 | nd |
| Infrared | >780 | nd |

nd is not detected by the eye.

Table 3
Spectrophotometric methods of colour determination in water and wastewater (APHA, 1998; Hao et al., 2000; Kao et al., 2001; Bisschops and Spanjers, 2003)

| Spectrophotometric method | Description |
|---|---|
| Tristimulus | Three tristimulus light filters combined with a specific light source (i.e. tungsten lamp) and a photoelectric cell inside a filter photometer. The output transmittance values are then converted to trichromatic coefficients and colour characteristic values |
| ADMI tristimulus | The ADMI colour value provides a true watercolour measure, which is independent of hue. Depending on the number of wavelengths chosen to calculate the ADMI value, this method can be differentiated in 3 (WL) ADMI, i.e. the transmittance is recorded at 590, 540 and 438 nm; or 31 (WL) ADMI, i.e. the transmittance is determined each 10 nm in the range of 400–700 nm |
| Spectra record | The complete spectrum is recorded in which the entire spectrum, or a part of it, can be used for comparison. A modified method has been suggested in which areas beneath an extinction curve represent the colour intensity, being expressed as space units |

Before measuring colour, the turbidity of the wastewater sample must be removed and the pH must be adjusted to 7.6.

methodologies, of which the most commonly used are Tristimulus Filter Method, American Dye Manufacturer Institute (ADMI) Tristimulus Filter Method, and Spectra record. A very good review on the measurements of both coloured wastewaters and the azo dye products, aromatic amines, by UV spectrophotometric detection, is reported elsewhere (Pinheiro et al., 2004).

## 4. Decolourisation processes

In the following items we will try to cover the biological and non-biological processes for dye decolourisation. It is worth to mention that this review gives a special attention to the azo dyes, because they represent the largest class of dyes used in industries, and due to the broad literature available.

### 4.1. Biological colour removal by aerobes

#### 4.1.1. Bacteria

Aromatic compounds are susceptible to biological degradation under both aerobic and anaerobic conditions (Field et al., 1995). Under aerobic conditions, the enzymes mono- and di-oxygenase catalyse the incorporation of oxygen from $O_2$ into the aromatic ring of organic compounds prior to ring fission (Madigan et al., 2003). In most monooxygenases, the electron donor is NADH or NAD(P)H, even though the direct coupling to $O_2$ is through a flavin that is reduced by the NADH or NAD(P)H donor (Madigan et al., 2003). Although azo dyes are aromatic compounds, their substituents containing mainly nitro and sulfonic groups, are quite recalcitrant to aerobic bacterial degradation (Claus et al., 2002). This fact is probably related either to the electron-withdrawing nature of the azo bond and their resistance to oxygenases attack, or because oxygen is a more effective electron acceptor, therefore having more preference for reducing equivalents than the azo dye (Chung et al., 1992; Knackmuss, 1996). However, in the presence of specific oxygen-catalysed enzymes called azo reductases, some aerobic bacteria are able to reduce azo compounds and produce aromatic amines (Stolz, 2001). Examples of aerobic azo reductases were found in *Pseudomonas* species strains K22 and KF46 (Zimmermann et al., 1982, 1984). These enzymes, after purification, characterisation and comparison were shown to be flavin-free. The aerobic azo reductases were able to use both NAD(P)H and NADH as cofactors and reductively cleaved not only the carboxylated growth substrates of the bacteria but also the sulfonated structural analogues. Recently, Blumel and Stolz (2003) cloned and characterized the genetic code of the aerobic azo reductase from *Pagmentiphaga kullae* K24. This strain was able to grow with the carboxylated azo compound 1-(4′-carboxyphenylazo)-4-naphtol as a sole source of carbon and energy. Furthermore, the gene encoded a protein with a molecular weight of 20,557 Da, having conserved a putative NAD(P)H-binding site in the amino-terminal region.

#### 4.1.2. Fungi

The capacity of fungi to reduce azo dyes is related to the formation of exoenzymes such as peroxidases and phenoloxidases. Peroxidases are hemoproteins that catalyse reactions in the presence of hydrogen peroxide (Duran et al., 2002). Lignin and manganese peroxidases have a similar reaction mechanism that starts with the enzyme oxidation by $H_2O_2$ to an oxidized state during their catalytic cycle. Afterwards, in a mechanism involving two successive electron transfers, substrates such as azo dyes reduce the enzyme to its original form (Stolz, 2001). Eighteen fungal strains able to degrade lignocellulosic material or lignin derivatives were tested with the azo dyes Reactive Orange 96, Reactive Violet 5 and Reactive Black 5. Only the strains of *Bjerkandera adusta*, *Trametes versicolor* and *Phanerochaete chrysosporium* were able to decolourise all azo dyes (Heinfling et al., 1997). Although lignin peroxidases are able to oxidize both phenolic and non-phenolic aromatic compounds, manganese peroxidases must convert $Mn^{2+}$ to $Mn^{3+}$ in order to oxidize phenolic compounds (Glenn et al., 1986). Phenoloxidases, which can be divided into tyrosinases and laccases, are oxidoreductases that can catalyse the oxidation of phenolic and other aromatic compounds without the use of cofactors (Duran et al., 2002). Laccases

are copper-containing enzymes that have a very broad substrate specificity with respect to electron donors, e.g. dyes (Abadulla et al., 2000). However, despite the fact that laccases from *T. versicolor*, *Polyporus pinisitus* and *Myceliophthora thermophila* were found to decolourise anthraquinone and indigoid-based dyes at high rates, the azo dye Direct Red 29 (Congo Red) was a very poor substrate for laccases (Claus et al., 2002). Chivukula and Renganathan (1995) cited that the azo dye must be electron-rich to be susceptible to oxidation by laccase of *Pyricularia oryzae*. This situation is suitable for the generation of a phenoxy radical, with consequent azo bond cleavage, and the release of molecular nitrogen (Fig. 3). The addition of redox mediators has been shown to further extend the substrate specificity of laccases with regard to several dye classes, although redox mediators can also be formed from laccase oxidation of phenolic azo dyes (Li et al., 1999; Soares et al., 2001; Claus et al., 2002).

### 4.2. Biological colour removal by strictly anaerobes or facultative microorganisms incubated under anaerobic conditions

Under anaerobic conditions a low redox potential (<−50 mV) can be achieved, which is necessary for the effective decolourisation of dyes (Beydilli et al., 1998; Bromley-Challenor et al., 2000). Colour removal under anaerobic conditions is also referred as *dye reduction* in which literature mostly covers the biochemistry of azo dye reduction. The azo bond cleavage –N=N– involves a transfer of four-electrons (reducing equivalents), which proceeds through two stages at the azo linkage. In each stage two electrons are transferred to the azo dye, which acts as a final electron acceptor:

Anaerobic conditions

$$(R_1\text{–}N{=}N\text{–}R_2) + 2e^- + 2H^+ \rightarrow R_1\text{–}HN\text{–}NH\text{–}R_2$$

(Hydrazo intermediate)

$$(R_1\text{–}HN\text{–}NH\text{–}R_2) + 2e^- + 2H^+ \rightarrow R_1\text{–}NH_2 + R_2\text{–}NH_2$$

(Reductive cleavage of the azo bond)

The exact mechanism of azo dye reduction, whether occurring intracellularly or extracellularly, is still a subject of investigation, as is the role of biogenic intracellular water-soluble electron carriers such as flavins. Reduced flavins can act as an electron shuttle from nicotinamide adenine dinucleotide phosphate (NADPH)-dependent flavoproteins to azo dye as electron acceptor (Gingell and Walker, 1971). Intracellular azo dye reduction cannot be responsible for the conversion of all types of azo dyes, especially for sulfonated azo dyes, which have limited membrane permeability (Stolz, 2001). Kudlich et al. (1997) demonstrated an increase on colour removal rates of sulfonated azo dyes by cell free-extracts, as well as after addition of toluene, i.e. a membrane-active compound which increases cell lysis, thus showing the limited membrane permeability of this type of dye. The current hypothesis is that

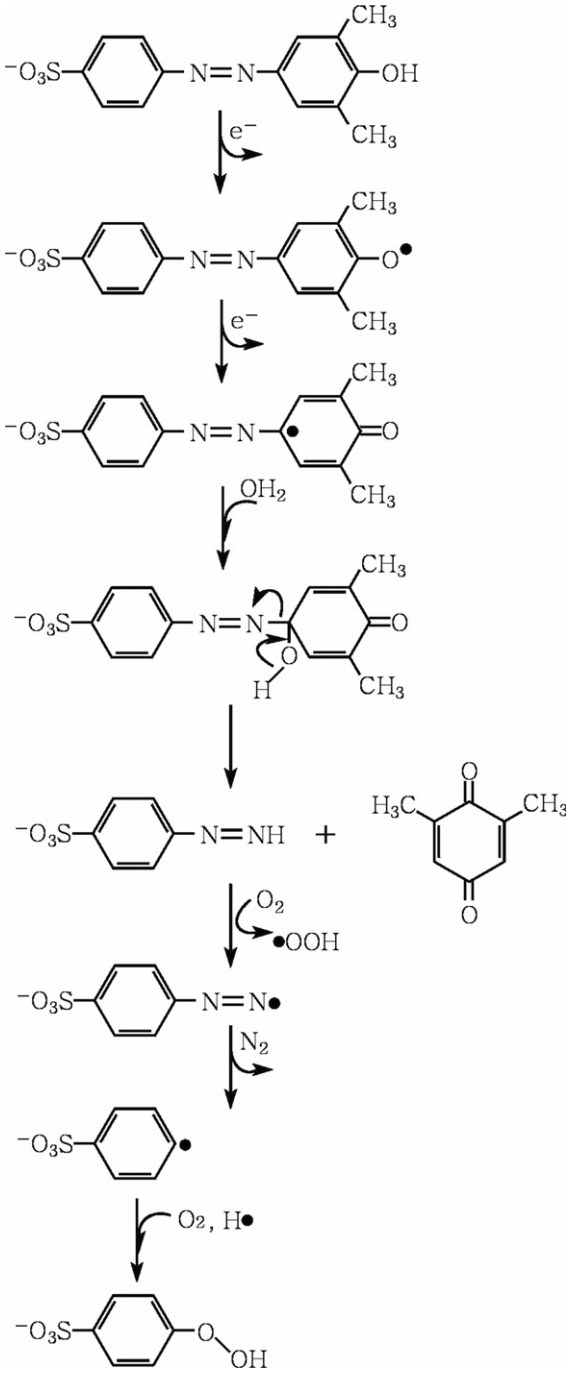


Fig. 3. Proposed pathway for the peroxidases-catalysed degradation of 4-(4′-sulfophenylazo)-2,6-dimethylphenol by *Pyricularia oryzae* (Chivukula and Renganathan, 1995).

azo dye reduction mostly occurs by extracellular or membrane-bond enzymes (Stolz, 2001). Reduced cytoplasmatic cofactors such as reduced flavins do not contribute to the chemical dye reduction due to their inability to cross living cell membranes (Russ et al., 2000). However, cell fractionation experiments demonstrated that a quinone reductase activity located in the cell membranes enhanced the reductive decolourisation of a sulfonated azo compound, and no dye cross-membrane transport was required (Kudlich et al., 1997). Recently, a NADH-dependent lawsone reduc-

tase activity located in the cytosolic fraction of *Escherichia coli* also showed the capacity for azo dye reduction (Rau and Stolz, 2003).

*4.2.1. Biological and chemical reductive decolourisation*

The reductive decolourisation of azo dyes under anaerobic conditions is a combination of both biological and chemical mechanisms. The *biological* contribution can be divided in specialised enzymes called azo reductases, which are present in bacteria that are able to grow using only azo dye as a carbon and energy source. However, up to date there is no clear evidence of anaerobic azo reductase; or non-specific enzymes that catalyse the reduction of a wide range of electron-withdrawing contaminants, including azo dyes (Stolz, 2001). Thus, a co-metabolic reaction is probably the main mechanism of dye reduction (Fig. 4), in which the reducing equivalents or reduced cofactors like NADH, NAD(P)H, $FMNH_2$ and $FADH_2$ acting as secondary electron donor, channel electrons to cleave the azo bond (Gingell and Walker, 1971).

The *chemical* contribution to the reductive decolourisation of azo dyes under anaerobic conditions may involve biogenic reductants like sulphide, cysteine, ascorbate or $Fe^{2+}$ (Yoo, 2002; Van der Zee et al., 2003). Particularly for sulphide, it can be formed by sulphate reduction in anaerobic bioreactors. Therefore, there will be a competition between sulphate and dye to become the terminal electron acceptor of the reducing equivalents. Van der Zee et al. (2003) observed that different sulphate concentrations did not have an adverse effect on the reduction of RR2 in either batch assays or reactor experiments. The authors concluded that sulphate, even present at concentrations up to 60 mM, did not obstruct the transfer of electron to the azo dye. However, any noticeable effect of sulphate was verified on the dye removal efficiency in the lab experiment. In another investigation on colour removal, Albuquerque et al. (2005) used an anaerobic–aerobic sequencing batch reactor fed with sulphate (0.35 mM). The results indicated that the decolourisation capacity was not improved while testing the dye Acid Orange 7, even though a sulphate reducing microbial population was established. Therefore, for a real perspective application, the contribution of sulphide generated by sulphate reduction seems to be negligible, and therefore the colour removal is mainly due to biological processes.

Fig. 5 shows the electron flow preference in the presence of different redox couples involved in biological processes.

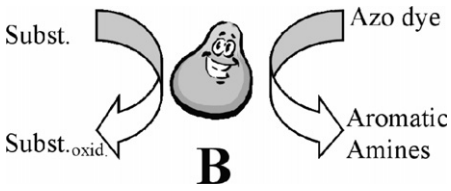


Fig. 4. Schematic for direct enzymatic azo dye reduction. Legend: Subst., substrate or primary electron donor; $Subst._{oxid}$, products of substrate oxidation.

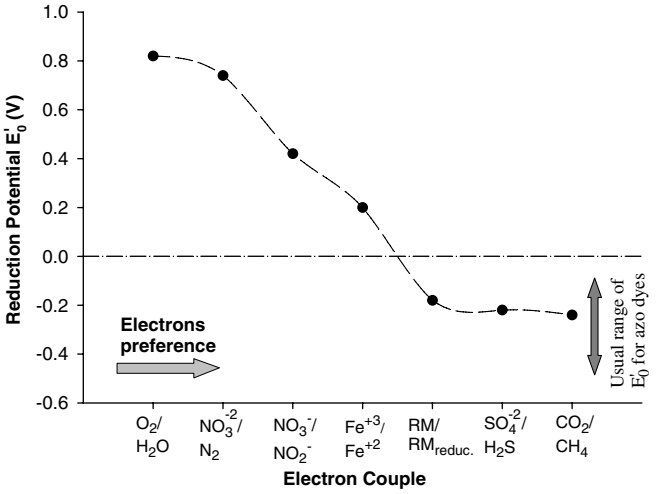


Fig. 5. Electron flow preference as a function of the different electron couples (adapted from Dubin and Wright (1975); Cervantes (2002); Madigan et al. (2003); Dos Santos (2005)). RM and $RM_{reduc}$ are the oxidized and the reduced form of the redox mediator, respectively.

Thus, oxygen is a more effective electron acceptor than azo dyes, which justify the low decolourisation rates (10–30%) under aerobic conditions.

Nevertheless, either by using pure cultures or granular sludge under anaerobic conditions, literature reports poor reductive decolourisation with specific dyes (Brown and DeVito, 1993; Van der Zee et al., 2001b; Dos Santos et al., 2005b,c). Furthermore, the rates are extremely dependent on the type of dye, in which the azo dyes generally present the highest rates of decolourisation. On the other hand, anthraquinone and phthalocyanine dyes are shown to be rather recalcitrant (Dos Santos et al., 2005a; Lee and Pavlostathis, 2004; Lee et al., 2005, 2006). Another drawback is that some dyes are quite toxic to the anaerobic microorganisms, which in some cases, may lead to a permanent loss of the methanogenic activity even for low dye concentrations (Brown and Laboureur, 1983a; Van der Zee et al., 2001a; Fontenot et al., 2003; Van der Zee and Villaverde, 2005; Lee et al., 2006). For instance, the inhibitory concentration of RB19, exerting 50% reduction in methanogenic activity (IC-value), was 55 mg/l at 30 °C (Dos Santos et al., 2005a).

Table 4 shows the decolourisation of some dyes by using anaerobic granular sludge under mesophilic conditions. It is important to mention that a sound comparison among investigations is extremely difficult because of the differences in type and concentrations of dyes, sludge source and concentrations, electron donor, the way of calculating the decolourisation rates, etc.

*4.2.2. Reductive decolourisation of azo dyes in the presence of redox mediators*

Redox mediators are compounds that accelerate the electron transfer from a primary electron donor to a terminal electron acceptor, which may increase the reaction rates

Table 4
Colour removal by anaerobic granular sludge under mesophilic conditions

| Type | Name | Decolouration (%) | Decolouration rates | Comments | Ref. |
|---|---|---|---|---|---|
| Anthraquinone | Reactive Blue 19 | 70 | – | About 50 mg/l of dye | 1 |
| Anthraquinone | Acid Blue 80 | 7 | – | About 50 mg/l of dye | 1 |
| Anthraquinone | Acid Blue 25 | 67 | – | About 50 mg/l of dye | 1 |
| Anthraquinone | Basic Blue 22 | 62 | – | About 50 mg/l of dye | 1 |
| Anthraquinone | Reactive Blue 4 | 57 | 13.4 mg/l/h | About 50 mg/l (0.08 mM) of dye | 2 |
| Anthraquinone | Reactive Blue 19 | 99 | 14.6 mg/l/h | About 50 mg/l (0.08 mM) of dye | 2 |
| Anthraquinone | Reactive Blue 4 | 73 | 4.3 mg/l/h | About 50 mg/l (0.08 mM) of dye | 3 |
| Anthraquinone | Reactive Blue 19 | 90 | 13.0 mg/l/h | About 50 mg/l (0.08 mM) of dye | 3 |
| Anthraquinone | Reactive Blue 5 | 37 | – | | 4 |
| Anthraquinone | Reactive Blue 49 | 9 | – | | 5 |
| Anthraquinone | Acid Blue 25 | 68 | – | | 6 |
| Anthraquinone | Disperse Red 159 | 0 | 0 | | 7 |
| Anthraquinone | Disperse Blue 56 | 0 | 0 | | 8 |
| Anthraquinone | Reactive Blue 4 | >84 | – | About 300 mg/l of dye | 9 |
| Anthraquinone | Reactive Blue 19 | >84 | – | About 300 mg/l of dye | 9 |
| Anthraquinone | Reactive Blue 19 | 87 | – | About 1032 mg/l | 10 |
| Azo dye | Acid Orange 7 | 99 | 1.49 $day^{-1}$ | About 0.3 mM of dye | 11 |
| Azo dye | Acid Red 266 | 95 | 0.20 $day^{-1}$ | About 0.3 mM of dye | 11 |
| Azo dye | Acid Yellow 137 | 95 | 0.35 $day^{-1}$ | About 0.3 mM of dye | 11 |
| Azo dye | Acid Yellow 159 | 97 | 0.72 $day^{-1}$ | About 0.3 mM of dye | 11 |
| Azo dye | Basic Red 23 | 99 | 10.00 $day^{-1}$ | About 0.3 mM of dye | 11 |
| Azo dye | Direct Black 19 | 99 | 3.00 $day^{-1}$ | About 0.3 mM of dye | 11 |
| Azo dye | Direct Blue 53 | 99 | 0.24 $day^{-1}$ | About 0.3 mM of dye | 11 |
| Azo dye | Direct Blue 71 | 100 | 0.61 $day^{-1}$ | About 0.3 mM of dye | 11 |
| Azo dye | Direct Red 79 | 97 | 16.60 $day^{-1}$ | About 0.3 mM of dye | 11 |
| Azo dye | Direct Red 81 | 99 | 7.80 $day^{-1}$ | About 0.3 mM of dye | 11 |
| Azo dye | Direct Yellow 4 | 95 | 1.03 $day^{-1}$ | About 0.3 mM of dye | 11 |
| Azo dye | Direct Yellow 12 | 86 | 1.17 $day^{-1}$ | About 0.3 mM of dye | 11 |
| Azo dye | Direct Yellow 50 | 99 | 2.00 $day^{-1}$ | About 0.3 mM of dye | 11 |
| Azo dye | Mordant Orange 1 | 97 | 1.74 $day^{-1}$ | About 0.3 mM of dye | 11 |
| Azo dye | Mordant Yellow 10 | 95 | 1.86 $day^{-1}$ | About 0.3 mM of dye | 11 |
| Azo dye | Reactive Black 5 | 99 | 5.00 $day^{-1}$ | About 0.3 mM of dye | 11 |
| Azo dye | Reactive Orange 14 | 98 | 0.17 $day^{-1}$ | About 0.3 mM of dye | 11 |
| Azo dye | Reactive Orange 16 | 97 | 2.10 $day^{-1}$ | About 0.3 mM of dye | 11 |
| Azo dye | Reactive Red 2 | 100 | 0.31 $day^{-1}$ | About 0.3 mM of dye | 11 |
| Azo dye | Reactive Red 4 | 99 | 0.45 $day^{-1}$ | About 0.3 mM of dye | 11 |
| Azo dye | Reactive Yellow 2 | 73 | 0.01 $day^{-1}$ | About 0.3 mM of dye | 11 |
| Azo dye | Reactive Red 235 | 100 | 4.42 $day^{-1}$ | About 50 mg/l | 12 |
| Azo dye | Reactive Blue 235 | 100 | 23.5 $day^{-1}$ | About 50 mg/l | 12 |
| Azo dye | Reactive Yellow 168 | 100 | 23.4 $day^{-1}$ | About 50 mg/l | 12 |
| Azo dye | Reactive Red 198 | 95 | 11.6 mg/l/h | About 300 mg/l of dye | 13 |
| Azo dye | Mordant Blue 13 | 83 | – | About 50 mg/l of dye | 1 |
| Azo dye | Mordant Black 9 | 77 | – | About 50 mg/l of dye | 1 |
| Azo dye | Basic Red 18 | 92 | – | About 50 mg/l of dye | 1 |
| Azo dye | Acid Yellow 151 | 88 | – | About 50 mg/l of dye | 1 |
| Azo dye | Direct Red 7 | 92 | – | About 50 mg/l of dye | 1 |
| Azo dye | Acid Red 114 | 62 | – | About 50 mg/l of dye | 1 |
| Azo dye | Direct Blue 15 | 83 | – | About 50 mg/l of dye | 1 |
| Azo dye | Direct Yellow 12 | 75 | – | About 50 mg/l of dye | 1 |
| Azo dye | Reactive Black 5 | 81 | – | About 50 mg/l of dye | 1 |
| Azo dye | Acid Blue 113 | 94 | – | About 50 mg/l of dye | 1 |
| Azo dye | Direct Black 19 | 51 | – | About 50 mg/l of dye | 1 |
| Azo dye | Direct Black 22 | 61 | – | About 50 mg/l of dye | 1 |
| Methine | Basic Yellow 28 | 35 | – | About 50 mg/l of dye | 1 |
| Nitro | Acid Orange 3 | 62 | – | About 50 mg/l of dye | 1 |
| Oxazine | Basic Blue 3 | 62 | – | About 50 mg/l of dye | 1 |
| Phthalocyanine | Reactive Blue 21 | 80 | 8.6 mg/l/h | About 300 mg/l of dye | 13 |
| Phthalocyanine | Reactive Blue 21 | 36 | – | About 50 mg/l of dye | 1 |
| Phthalocyanine | Reactive Blue 7 | 49–66 | | About 300 mg/l of dye | 9 |
| Phthalocyanine | Reactive Blue 21 | 49–66 | | About 300 mg/l of dye | 9 |
| Phthalocyanine | Reactive Blue 21 | 37 | | About 519 mg/l of dye | 10 |

[1]: Brown and Laboureur (1983b); [2] Fontenot et al. (2002); [3] Lee and Pavlostathis (2004); [4] Luangdilok and Paswad (2000); [5] Carliell et al. (1994); [6] Brown and Hamburger (1987); [7] Malpei et al. (1998); [8] Delée et al. (1998); [9] Lee et al. (2006); [10] Lee et al. (2005); [11] Van der Zee et al. (2001b); [12] Willetts and Ashbolt (2000); [13] Fontenot et al. (2003).

by one to several orders of magnitude (Cervantes, 2002; Dos Santos, 2005). Redox mediators have shown to be effective not only for reductive decolourisation, but also for the reductive transformation of many contaminants such as iron (Lovley et al., 1998), nitroaromatics (Dunnivant et al., 1992), polyhalogenated compounds (O'Loughlin et al., 1999) and radionuclides (Fredrickson et al., 2000). Recently it was found that during the aerobic degradation of naphthalene-2-sulfonate (2NS) by *Sphingomonas xenophaga* strain BN6, quinoid redox mediators were produced, which mediated the reduction of azo dye under anaerobic conditions (Keck et al., 2002). Flavin-based compounds like FAD, FMN and riboflavin, as well as quinone-based compounds like AQS, AQDS and lawsone, have been extensively reported as redox mediators during azo dye reduction (Semdé et al., 1998; Cervantes et al., 2000; Rau et al., 2002a; Field and Brady, 2003; Dos Santos et al., 2004a, 2005b; Encinas-Yocupicio et al., 2006). Reductive decolourisation of azo dyes in the presence of redox mediators occurs in two distinct steps, the first step being a non-specific enzymatic mediator reduction, and the second step being a chemical reoxidation of the mediator by the azo dyes (Fig. 6) (Keck et al., 1997).

Theoretically, feasible redox mediators for biological azo dye reduction must have redox potentials between the half reactions of the azo dye and the primary electron donor (Van der Zee et al., 2003). Unfortunately, the standard redox potential ($E_0'$) for most azo dyes is unknown, but this information can be obtained by using polarography. In a screening of redox potential values for different azo dyes, it was found that $E_0'$ values are generally between −0.430 and −0.180 V (Dubin and Wright, 1975). Rau et al. (2002a,b) cite that the NAD(P)H cofactor, which has the lowest $E_0'$ value of −0.320 V, seems to set the limits of redox mediators application. The reason for this is that mediators with a more negative $E_0'$ value will not be reduced by the cells, and mediators with $E_0'$ greater than −0.05 V will not efficiently reduce the azo bond at high rates. Fig. 7 shows the $E_0'$ values for both quinone-based and non-quinone-based redox mediators.

The standard redox potential value ($E_0'$) is a good indication of a compound capacity to function as a redox mediator. However, apparently other factors are of importance as well since different decolourisation rates in the presence of mediators with similar $E_0'$ values have been reported, and similar decolourisation rates with mediators

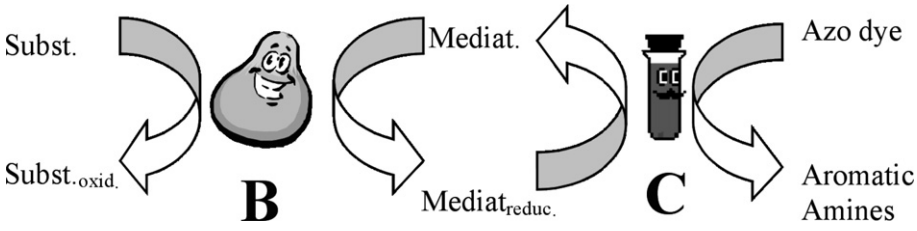


Fig. 6. Schematic for indirect azo dye reduction. B and C are the biological and chemical steps, respectively.

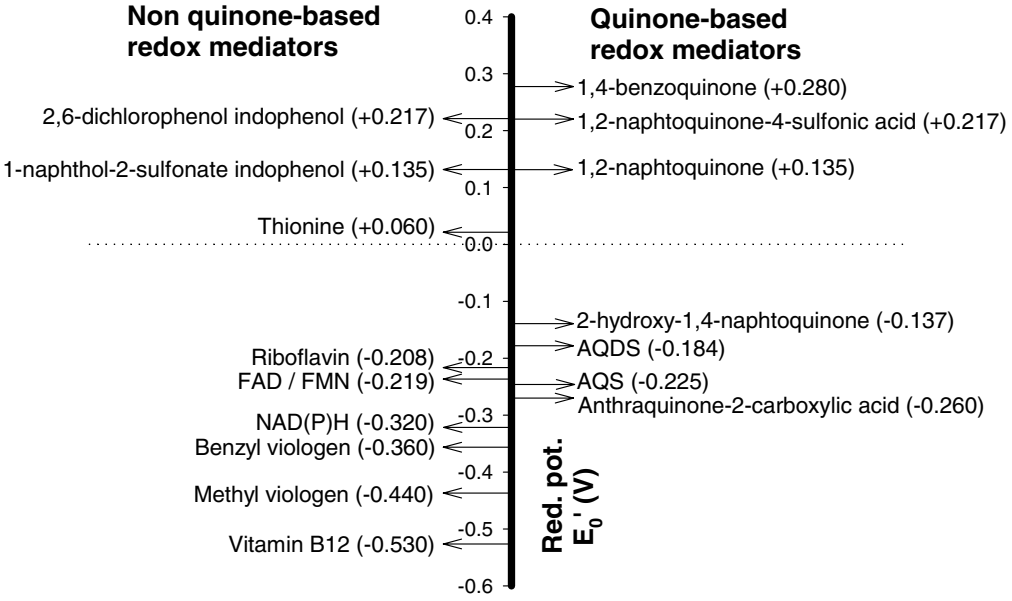


Fig. 7. $E_0'$ values both for quinone-based redox mediators and non-quinone-based redox mediators (adapted from Rau et al. (2002a); Fultz and Durst (1982)).

with different $E'_0$ values (Dos Santos et al., 2004). For instance, Brown (1981) tested the polymeric nitro dye Poly Y-607 and found that methyl viologen and benzyl viologen increased the decolourisation rates 4.5-fold, even though the $E'_0$ of methyl viologen is much lower than that of benzyl viologen, i.e. −0.440 V and −0.360 V, respectively (Fig. 7). Walker and Ryan (1971) postulated that decolourisation rates are related to the electron density in the azo bond region. They suggested that colour removal rates would increase by lowering the electron density in the azo linkage. Therefore, the use of redox mediators would not only tend to accelerate the transfer of reducing equivalents to the terminal electron acceptor, i.e. the azo dye, but also to minimize the steric hindrance of the dye molecule (Bragger et al., 1997; Moir et al., 2001) and to decrease the activation energy of the chemical reaction (Dos Santos, 2005). Thus, in estimating the theoretical decolourisation rates by using specific redox mediators, differences in electro-chemical factors between mediator and azo dye should also be considered.

#### 4.2.3. Microbiological aspects of the reductive decolourisation of azo dyes

*4.2.3.1. Pure cultures.* The literature extensively reports the use of pure cultures (Table 5), either whole cells or specific enzymes, for a better insight of the anaerobic azo dye reduction mechanisms, which are not fully understood yet (Stolz, 2001; Pearce et al., 2003, 2006; Dos Santos et al., 2006b).

Microbial decolourisation requires an unspecific enzymatic capacity ubiquitously found in a wide diversity of microorganisms (Chung and Stevens, 1993). This has been mainly demonstrated with microorganisms present in the intestine such as *Clostridium*, *Salmonella*, *Bacillus*, *Eubacterium* and *Escherichia coli*, which are able to reduce the dyes ingested through food, drugs and cosmetics (Brown and DeVito, 1993; Rau et al., 2002b; Chen et al., 2004). The understanding of azo dye reduction mechanisms is important not only under a biotechnological approach toward the use of biological processes for decolourisation, but also under a medical approach to have an insight into how the intestinal microflora metabolites the ingested azo dyes (Brown and DeVito, 1993; Semdé et al., 1998). Azo dyes are converted into aromatic amines because of both the presence of microflora and the anaerobic condition found in the human intestine. Aromatic amines present a mutagenic and carcinogenic character much higher than their precursor azo dyes (Weisburger, 2002). Therefore, a lot of effort has been made in the production of compounds, which are resistant to these reductive transformations. Another approach has been investigated in the use of azo polymers that would be insoluble in the upper gastrointestinal track, but susceptible to degradation on the colon, acting as an oral colon-specific drug delivery system (Bragger et al., 1997; Rau et al., 2002b). In this review we focus on the biotechnological approach of azo dye reduction.

*4.2.3.2. Granular sludge.* Even though anaerobic azo dye reduction could be readily achieved with different microorganisms, there is no strain reported so far that is able to decolourise a broad range of azo dyes. Therefore, the use of a specific strain or enzymes on reductive decolourisation does not make much sense in treating textile wastewater, which is composed of many kinds of dyes (Laszlo, 2000). The use of mixed cultures such as anaerobic granular sludge, which is composed of stable microbial pellets with a high activity, is probably a more logic alternative. Different reactor configurations like the widely used upflow anaerobic sludge bed (UASB) system and expanded granular sludge bed (EGSB) system, are used to immobilize high concentrations of biomass (Lettinga et al., 1980; Lettinga, 1995; van Lier et al., 2001). Indeed, the different microbial consortia present in anaerobic granular sludge can carry out tasks that no individual pure culture can undertake successfully (Nigam et al., 1996; Pearce et al., 2003).

However, little is known about the microbiological aspects of the reductive decolourisation of azo dyes with anaerobic consortia commonly found in wastewater treatment plants, although the applicability of the cost-effective high-rate anaerobic reactors for azo dye reduction has been well demonstrated (Cervantes et al., 2001; Dos Santos et al., 2003, 2004b, 2006b). A very complete review on bioreactor studies in lab scale for dye decolourisation was recently published elsewhere (Van der Zee and Villaverde, 2005). However, literature lacks decolourisation studies conducted in full scale. Frijters et al. (2006) reported a success example in Nijverdal (The Netherlands) of a combined anaerobic/aerobic treatment of a textile wastewater containing both soluble and insoluble dyes. Efficiencies of about 80–95% on colour removal were found, in which the anaerobic bioreactor was the main responsible in decolourising and detoxifying the textile wastewater.

As previously explained, the reductive decolourisation of azo dyes by using methanogenic anaerobic granular sludge is very likely controlled by a co-metabolic reaction in the presence of different electron donors, in which the azo dye is the terminal electron acceptor of the reduced cofactors (Dos Santos, 2005). Thus, the reducing equivalents are formed during the conversion of the primary electron donor, i.e. the organic matter, during the different steps of carbon flow under anaerobic conditions (Fig. 8).

According to Gujer and Zehnder (1983), the organic matter is initially hydrolysed; namely, enzymes produced by fermentative bacteria hydrolyse complex molecules such as proteins, polysaccharides, nucleic acid and fats into their corresponding monomers, which are amino acids, sugars and long chain fatty acids. Afterwards, these monomers are fermented to reduced organic compounds like short chain fatty acids, alcohols and lactate in a step called acidogenesis. Subsequently, these acids can either be converted into $H_2/CO_2$ by hydrogen-forming microorganisms or into acetate by acetate-forming microorganisms. Acetate can also be formed via $H_2/CO_2$ pathway in a step called homoacetogenesis. Finally, the final product methane/$CO_2$ can

Table 5
Examples of facultative and strictly anaerobic bacterial cultures, which were able to decolourise azo dyes under anaerobic conditions

| Organism | Dyes | Activity | Decolouration (%) | Comments | Ref. |
|---|---|---|---|---|---|
| *Clostridium perfringens* ATCC 3626 | | μmol/ml/h | | Dye concentration of 0.033 mM | 1 |
| | Amaranth | 0.74 | – | | |
| | Methyl Orange | 0.62 | – | | |
| | Orange II | 0.70 | – | | |
| | Tartrazine | 0.67 | – | | |
| *Bacteroides fragilis* | | μmol/ml/h | | After 6 h of incubation. Dye concentration of 0.1 mM | 2 |
| | Amaranth | 0.30 | 66.0 | | |
| | Orange II | 0.20 | 37.0 | | |
| | Tartrazine | 0.08 | 9.0 | | |
| *Pseudomonas* GM3 | Acid Violet 7 | – | 97.4 | After 72 h of incubation. Dye concentration of 100 mg/l | 3 |
| | Reactive Blue 2 | – | 18.3 | | |
| | Acid Green 27 | – | 75.6 | | |
| | Acid Red 183 | – | 20.1 | | |
| | Indigo Carmine | – | 69.0 | | |
| *Sphingomonas xenophaga* BN6 | | μmol/min/g protein | | Dye concentration of 0.1 mM | 4 |
| | Acid Red 27 | 0.10 | – | | |
| | Acid Orange 20 | 0.10 | – | | |
| | Acid Orange 7 | 0.30 | – | | |
| | Acid Red 14 | 0.20 | – | | |
| | Acid Yellow 23 | 0.10 | – | | |
| | Acid Black 1 | 0.30 | – | | |
| *Enterococcus faecalis* | | $AU \times 10^{-2}$/mg protein | | After 20 h of incubation. Dye concentration of 0.2 mM | 5 |
| | Methyl Red | 1.81 | 99.4 | | |
| | Orange II | 1.39 | 95.1 | | |
| | Orange G | 1.20 | 64.1 | | |
| | Amaranth | 1.37 | 99.5 | | |
| *Eubacterium biforme* | Tartrazine | – | 4.0 | After 150 minutes of incubation. Dye concentration of 2 mM | 6 |
| | Sunset Yellow | – | 22.0 | | |
| | Methyl Orange | – | 79.0 | | |
| | Orange II | – | 81.0 | | |
| | Amaranth | – | 19.0 | | |
| | Allura Red 40 | – | 11.0 | | |

[1] Semdé et al. (1998); [2] Bragger et al. (1997); [3] Yu et al. (2001); [4] Rau et al. (2002a); [5] Chen et al. (2004); [6] Chung et al. (1978).

be formed in the methanogenesis step. Methanogens are able to directly use substrates like $H_2$, acetate, formate and methanol to produce methane.

Reductive decolourisation of azo dyes is extremely dependent on the type of primary electron donor. Acetate and other volatile fatty acids are normally poor electron donors, whereas ethanol, glucose, $H_2/CO_2$ and formate are more effective electron donors for dye reduction (Tan et al., 1999; Dos Santos et al., 2003, 2005b; Pearce et al., 2006). Donlon et al. (1997) reported that interspecies hydrogen resulting from the oxidation of substrates such as butyrate, propionate, and ethanol could provide the medium with reducing equivalents, and thus stimulation of the nitrophenol reduction. In the latter work, the direct methane precursors acetate and methanol did not stimulate the nitrophenol reduction rates. Similar results were also reported during the reductive dechlorination of carbon tetrachloride (CT) by anaerobic granular sludge in which acetate and methanol were marginally utilized to support CT dechlorination (Cervantes et al., 2004). The reason why acetate, methanol and VFAs are poor electron donors for reductive biotransformations is not clear yet. Nevertheless, a possible explanation is that the acetoclastic methanogens do not participate effectively on dye reduction, and the

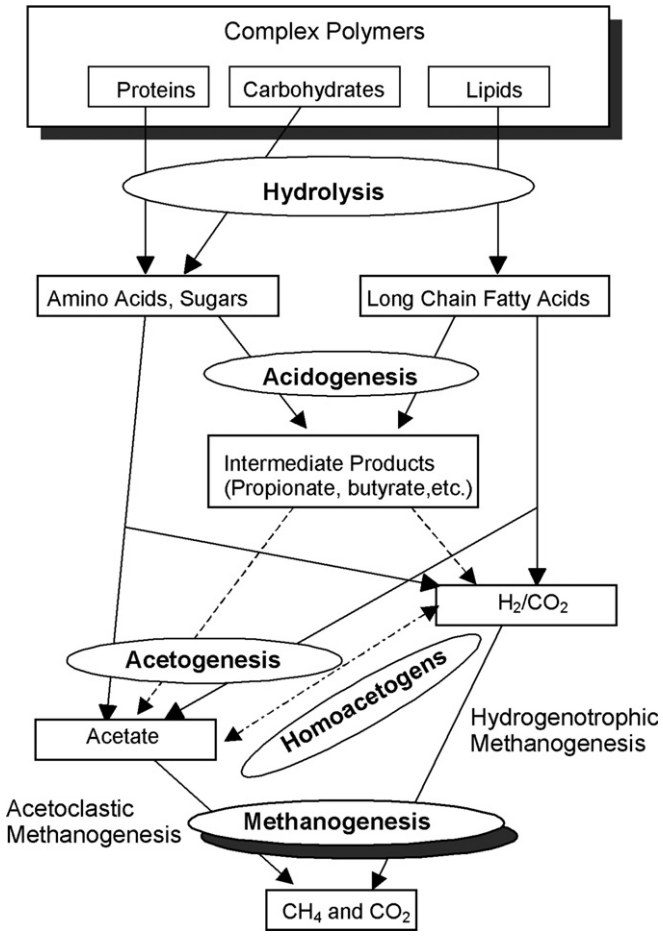


Fig. 8. Conversion of complex organic matter in methanogenic anaerobic reactors (adapted from Gujer and Zehnder (1983)).

main contribution is attributed to fermentative bacteria and hydrogenotrophic methanogens. Thus, probably VFAs are poor electron donors because they are mainly converted to acetate due to the thermodynamics reasons. In fact, there will be a competition for reducing equivalents between dye reducers and other microorganisms during all the steps of organic matter conversion under anaerobic conditions (Dos Santos et al., 2006b).

Dos Santos et al. (2006b) found that in the absence of the redox mediator riboflavin, the effect of the methanogenic inhibitor BES on azo dye reduction was negligible with the methanogenic substrates acetate and methanol. In contrast, remarkable results were obtained when hydrogen and formate were included as electron donors; the rates of decolourisation were considerably higher (up to 1.7-fold) in BES-supplemented bottles compared to non-inhibited controls. They concluded that apparently, the inclusion of BES stimulated azo dye reduction by chemically out-competing methanogenesis for the reducing equivalents available in hydrogen- and formate-supplemented cultures, and therefore acetate- and methanol utilizing methanogens do not play a role in the reductive decolourisation. The effect of BES with hydrogen as the electron donor is in agreement with the conclusions of Field et al. (2004), who studied the reduction of arsenate by a methanogenic consortium. In this study, methanogens and arsenate reducers were probably competing for the same electrons, likely in a similar reduction mechanism as we found for dye reduction.

The conversion of the electron donors, e.g. ethanol, glucose, used for both dye reduction and methanogenesis is only maintained if there is equilibrium among the syntrophic group of microorganisms present in the bioreactor (Fig. 8). The degree of dependence among these microorganisms varies considerably (Schink, 2002). Despite the fact that the last members in the bacterial food chain are dependent on the earlier ones, e.g. interdependence between methanogens and acetogens, they may be extremely important for the first organisms in the consumption chain by removing metabolic products (Schink, 1997). Dos Santos et al. (2005a) observed that the anthraquinone dye RB19 was mainly toxic to the acetate-utilising methanogens, whereas acidogens were not affected by the dye toxicity. In another study, Lee et al. (2006) found high and moderate decolourisation extend for the anthraquinone (over 84%) and phthalocyanine (49–66%) dyes, respectively, after prolonged incubations under anoxic/anaerobic conditions with different carbon sources, despite the inhibition of the methanogenic activity. Therefore, the feasibility of coupling, in the same bioreactor, dye reduction and complete electron donor conversion into $CH_4$ must be carefully attempted.

### 4.3. Non-biological colour removal

#### 4.3.1. Physical–chemical methods

In physical–chemical methods coagulant agents like ferric salts or aluminium polychloride are used to form flocs with the dyes, which are then separated by filtration or sedimentation. Polyelectrolyte can also be dosed during the flocculation phase to improve the flocs settleability (Vandevivere et al., 1998). The coagulation–flocculation method is one of the most widely used processes in textile wastewater treatment plants in many countries such as Germany and France. It can be used either as a pre-treatment, post-treatment, or even as a main treatment system (Gähr et al., 1994; Marmagne and Coste, 1996). Marmagne and Coste (1996) reported that coagulation–flocculation methods were successfully applied for colour removal of sulphur and disperse dyes, whereas acid, direct, reactive and vat dyes presented very low coagulation–flocculation capacity. On top of the problem of low colour removal efficiency with some dyes, physical–chemical methods demand large chemicals inputs, and produce high volumes of polluted sludge, which then must be treated (Robinson et al., 2001; Anjaneyulu et al., 2005).

#### 4.3.2. Chemical methods

Chemical oxidation typically involves the use of an oxidising agent such as ozone ($O_3$), hydrogen peroxide ($H_2O_2$) and permanganate ($MnO_4$) to change the chemical

composition of a compound or a group of compounds, e.g. dyes (Metcalf and Eddy, 2003). Among these oxidants, ozone is the most widely used because of its high reactivity with many dyes, usually providing good colour removal efficiencies (Alaton et al., 2002). In a process called selective oxidation, ozone can be designed in such a way that only –N=N– bond scission occurs, and biodegradable compounds remain non-oxidised (Boncz, 2002). However, disperse dyes and those insoluble in water represent a drawback for the process, as well as the high cost of ozone (Hassan and Hawkyard, 2002; Anjaneyulu et al., 2005). The usual low efficiency of both colour and COD removals of conventional chemical oxidation techniques have been overcome by the development of the so-called advanced oxidation processes (AOP). In this process, oxidizing agents such as $O_3$ and $H_2O_2$ are used with catalysts (Fe, Mn and $TiO_2$), either in the presence or absence of an irradiation source (Anjaneyulu et al., 2005). Consequently, an improvement in the generation and use of the free hydroxyl radical ($HO^{\cdot}$) is obtained, which may represent a rate increase of one to several orders of magnitude compared with normal oxidants in the absence of a catalyst (Ince and Tezcanli, 1999). Table 6 gives an indication of the oxidative power of the hydroxyl radical based on the electrochemical oxidation potential (EOP) capacity compared with other oxidants.

At present, many different combinations of these AOP have been investigated for colour removal, all of which are capable of producing the free hydroxyl radical (HO). The first example is a reaction called the *Fenton reaction*, in which hydrogen peroxide is added in an acid solution (pH 2–3) containing $Fe^{2+}$ ions:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO + HO^{-} \quad (1)$$

In comparison with ozonation, this method is relatively cheap and also presents high COD removal and decolourisation efficiencies (Van der Zee, 2002). The main process drawbacks are the high sludge generation due to the flocculation of reagents and dye molecules (Robinson et al., 2001), as well as the need for decreasing the bulk pH to acidic conditions. Hassan and Hawkyard (2002) reported that a pre-ozonation of coloured wastewaters prior to Fenton reaction not only considerably accelerated the overall colour removal rates, but also decreased the sludge generation.

In *$H_2O_2/UV$* process HO radicals are formed when water-containing $H_2O_2$ is exposed to UV light, normally in the range of 200–280 nm (Metcalf and Eddy, 2003). The $H_2O_2$ photolysis follows the reaction:

$$H_2O_2 + \text{UV} \quad (\text{or } h\nu, \lambda \approx 200\text{–}280 \text{ nm}) \rightarrow HO^{\cdot} + HO^{\cdot} \quad (2)$$

This process is the most widely used AOP technology for the treatment of hazardous and refractory pollutants present in wastewaters, mainly because no sludge is formed and a high COD removal in a short retention time is achieved (Safarzadeh et al., 1997). Additionally, it has been successfully applied for colour removal. For instance, more than 95% decolourisation was achieved in treating reactive, basic, acid and direct dyes at pH 5, whereas disperse and vat dyes were only partially decolourised (Yang et al., 1998). A comparative study between ozone and $H_2O_2$/UV was carried out in treating a concentrated reactive dyebath from a textile factory. The $H_2O_2$/UV system presented decolourisation rates close to those rates obtained with ozone but with a lower cost (Alaton et al., 2002). In some cases however, the $H_2O_2$/UV process presents low COD and colour removal efficiency due to inefficient use of UV light (mainly for highly coloured wastewaters) (Moraes et al., 2000), or because of the low molar extinction coefficient of $H_2O_2$ (specific oxidation capacity), requiring high dosages of the latter.

The UV-based methods in the presence of a catalyst, e.g. a semiconductive material such as $TiO_2$, have also shown to distinctly enhance colour removal (So et al., 2002; Grzechulska and Morawski, 2002). Thus, different combinations such as ozone/$TiO_2$, ozone/$TiO_2$/$H_2O_2$ and $TiO_2$/$H_2O_2$ have been investigated, but they are enormously influenced by the type of dye, dye concentration and pH (Galindo et al., 2000). Recently, the utilization of solar technologies instead of UV-based methods has been attracting attention (Wang, 2000).

Table 6
Oxidation capacity of different oxidants in terms of electrochemical oxidation potential (EOP) (adapted from Metcalf and Eddy (2003))

| Oxidizing agent | EOP (V) |
|---|---|
| Fluorine | 3.06 |
| Hydroxyl radical | 2.80 |
| Oxygen (atomic) | 2.42 |
| Ozone | 2.08 |
| Hydrogen peroxide | 1.78 |
| Hypochlorite | 1.49 |
| Chlorine | 1.36 |
| Chlorine dioxide | 1.27 |
| Oxygen (molecular) | 1.23 |

#### 4.3.3. Physical methods

*Filtration* methods such as ultrafiltration, nanofiltration and reverse osmosis have been used for water reuse and chemical recovery. In the textile industry these filtration methods can be used for both filtering and recycling not only pigment-rich streams, but also mercerising and bleaching wastewaters. The specific temperature and chemical composition of the wastewater determine the type and porosity of the filter to be applied (Porter, 1997). The main drawbacks of membrane technology are the high investment costs, the potential membrane fouling, and the production of a concentrated dyebath which needs to be treated (Robinson et al., 2001). The recovery of concentrates from membranes, e.g. recovery of the sodium hydroxide used in the mercerising step or sizing agents such as polyvinyl alcohol (PVA), can attenuate the treatment costs (Porter, 1997). Water reuse from dyebath effluents

Table 7
Advantages and drawbacks of some non-biological decolourisation processes applied to textile wastewaters (after Robinson et al. (2001))

| Physical/chemical methods | Method description | Advantages | Disadvantages |
|---|---|---|---|
| Fenton reagents | Oxidation reaction using mainly $H_2O_2$–Fe(II) | Effective decolourisation of both soluble and insoluble dyes | Sludge generation |
| Ozonation | Oxidation reaction using ozone gas | Application in gaseous state: no alteration of volume | Short half-life (20 min) |
| Photochemical | Oxidation reaction using mainly $H_2O_2$-UV | No sludge production | Formation of by-products |
| NaOCl | Oxidation reaction using $Cl^+$ to attack the amino group | Initiation and acceleration of azo-bond cleavage | Release of aromatic amines |
| Electrochemical destruction | Oxidation reaction using electricity | Breakdown compounds are non-hazardous | High cost of electricity |
| Activated carbon | Dye removal by adsorption | Good removal of a wide variety of dyes | Very expensive |
| Membrane filtration | Physical separation | Removal of all dye types | Concentrated sludge production |
| Ion exchange | Ion exchange resin | Regeneration: no adsorbent loss | Not effective for all dyes |
| Electrokinetic coagulation | Addition of ferrous sulphate and ferric chloride | Economically feasible | High sludge production |

has been successfully achieved by using reverse osmosis. However, a coagulation and micro-filtration pre-treatment was necessary to avoid membrane fouling (Vandevivere et al., 1998). A very good option would be to consider an anaerobic pre-treatment followed by aerobic and membrane post-treatments, in order to recycle the water.

*Adsorption* methods for colour removal are based on the high affinity of many dyes for adsorbent materials. Decolourisation by adsorption is influenced by some physical–chemical factors like dye-adsorbent interactions, adsorbent surface area, particle size, temperature, pH and contact time (Mattioli et al., 2002; Anjaneyulu et al., 2005). The main criteria for the selection of an adsorbent should be based on characteristics such as high affinity and capacity for target compounds and the possibility of adsorbent regeneration (Karcher et al., 2001). Activated carbon (AC) is the most common adsorbent and can be very effective with many dyes (Walker and Weatherley, 1997). However, its efficiency is directly dependent upon the type of carbon material used and the wastewater characteristics, i.e. types of dyes present in the stream (Robinson et al., 2001). Additionally, AC is relatively expensive and has to be regenerated offsite with losses of about 10% in the thermal regeneration process. In order to decrease the adsorbent losses during regeneration, new adsorbent materials have been tested for their ability for on-site regeneration. Karcher et al. (2001) studied alternative materials such as zeolites, polymeric resins, ion exchangers and granulated ferric hydroxide. It was found that zeolites and microporous resins were unsuitable due to their low sorption capacity. Although the ion exchangers provided good sorption capacity, regeneration was sometimes difficult. A number of low-cost adsorbent materials like peat, bentonite clay and fly ash, have been investigated on colour removal (Ramakrishna and Viraraghavan, 1997; Anjaneyulu et al., 2005). However, the efficiency of these materials varied with the dye class. For instance, fly ash presented high sorption affinity for acid dyes, whereas peat and bentonite presented high affinity for basic dyes. Table 7 shows the advantages and drawbacks of some non-biological decolourisation processes applied to textile wastewaters.

## 5. Conclusions

All the decolourisation methods described in this review have advantages and drawbacks, and their selection will depend on the wastewater characteristics like class and concentration of dye, pH, salinity and toxic compounds. Compared to individual bacterial cells or specific enzymes, anaerobic colour removal by granular sludge as a pre-treatment for coloured wastewaters from dyebath and rinsing steps seems to be a very attractive technology. Moreover, the use of redox mediators and/or thermophilic treatment to accelerate decolourisation rates in bioreactors is very promising, while ways to immobilize redox mediators in bioreactors or their recovery when continuously dosed, still represents a challenge.

Efficient post-treatment methods, e.g. aerobic biological post-treatment, ozone and fenton reagents, for the complete mineralization of the aromatic amines, which are formed in the anaerobic step, must be utilized. Therefore, the treatment of textile wastewater either to guarantee the emission requirements or to close the water cycle should be composed of a sequence of treatments, and each scenario should be analysed individually.

## Acknowledgement

This work was supported by "Conselho Nacional de Desenvolvimento Científico e Technológico – CNPq" (Project no 200488/01-5), an organization of the Brazilian Government for the development of Science and Technology.

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