Rev Environ Sci Biotechnol (2011) 10:125–137
DOI 10.1007/s11157-011-9228-9

MINI-REVIEW

Reduction of azo dyes by anaerobic bacteria: microbiological and biochemical aspects

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Published online: 19 January 2011

Abstract Azo dyes are recalcitrant pollutants commonly found in several industrial wastewaters, such as those originated from textile factories, which generally persist to biological transformation. Discharge of these effluents in open water bodies not only represents an aesthetic problem, but also may limit photosynthesis in aquatic plants. Furthermore, many azo dyes and products derived from their partial transformation in the environment (e.g. aromatic amines) may be toxic or carcinogenic. Biological wastewater treatment processes have emerged as promising technologies to remove azo dyes from industrial effluents and intensive research has been conducted during the last two decades in order to elucidate the mechanisms involved in the reductive decolourisation of azo dyes. The present work describes the main biochemical and microbiological aspects involved in the reductive decolourisation of azo dyes by anaerobic bacteria.

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Keywords Anaerobic decolourisation · Bacterial consortia · Biological wastewater treatment · Textile industry

1 Introduction

In 2000, it was estimated that 5×10^{10} kg of fibres were produced worldwide, which consumed more than 8×10^{8} kg of different dyes and pigments (Broadbent 2001). In 2005, the global market size for dyes, pigments and intermediaries was US$ 23 billion. In terms of total volume, the global dyestuff production was 3.4×10^{10} kg, which accounted for annual global sales of nearly US$ 6 billion (Dyes and Pigments 2010). Unfortunately, linked to the great economical benefits given by the textile sector, severe environmental problems have been created due to discharge or inadequate disposal of wastes. The most important contaminants released by the textile industry are dyes, which enter into the environment mainly via discharge of wastewaters.

To judge the relative share of the different dye classes in the wastewater of textile-processing industries, dye consumption data should be considered together with the degree of fixation of the different dye classes. Recent statistics on the global production and use of dyes and on the relative distribution among the different dye classes are not readily available.

Table 1 Estimated degree of fixation for different dye/fibre combinations (Van der Zee 2002)

Dye class	Fibre	Degree of fixation (%)	Loss to effluent (%)
Acid	Polyamide	80–95	5–20
Basic	Acrylic	95–100	0–5
Direct	Cellulose	70–95	0–10
Disperse	Polyester	90–100	2–10
Metal-complex	Wool	90–98	10–50
Reactive	Cellulose	50–90	10–40
Sulphur	Cellulose	60–90	5–20
Vat	Cellulose	80–95	

Table 1 summarises the estimated degree of fixation for different dye/fibre combinations, which indicate that approximately 75% of the dyes discharged by Western-Europe textile-processing industries belong to the classes of reactive (~36%), acid (~25%) and direct (~15%), all of which are dye classes primarily composed by azo dyes. In fact, azo dyes, which are characterised by the presence of one to several –N = N– chromophore groups in their structure, constitute the largest (~70% in weight) class of dyes applied in textile processing (Van der Zee 2002). The markets for the dyes are mainly dominated by reactive and disperse dyes. In fact, the demand for these two dyes is expected to grow in the future also. Nations like China, South Korea and Taiwan are strong players in the field of disperse dyes. Interestingly, India has taken lead in production of reactive dyes because of the availability of an intermediate called vinyl sulphone in the country (Dyes and Pigments 2010).

In recent years, new legislation has affected dyestuff producers' product portfolios. For instance, the European Commission banned many azo dyes in 2002 and navy blue (a chromate-based azo dye, one of the most widely used dyes in the leather industry) in June 2004. REACH (an EU regulation of chemicals), which was enacted in 2007, also affects consumption of synthetic dyes.

2 Environmental and eco-toxicological problems associated with the discharge of textile wastewaters

Most textile wastewaters are highly coloured because they are typically discharged with a dye contain in the range 10–200 mg/L and many dyes are visible in water at concentrations as low as 1 mg/L (O′Neill et al. 1999; Pandey et al. 2007). Thus, discharge of these effluents in open water bodies not only represents an aesthetic problem, but also may limit photosynthesis in aquatic plants. As dyes are designed to be chemically and photolytically stable, they are highly persistent in natural environments. 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). The release of dyes may therefore present eco-toxic hazard and introduces the potential danger of bioaccumulation that may eventually affect man by transport through the food chain (Dos Santos et al. 2006a).

2.1 Bioaccumulation

Intensive research has documented the bioaccumulation tendency of dyestuffs in fish. The bio-concentration factors (BCF's) of 75 dyes from different application classes were determined and related to the partition coefficient *n*-octanol/water (K_{OW}) of each different compound. Water-soluble dyes with low K_{OW} (e.g. ionic dyes like acid, reactive and basic dyes) did not bio-accumulate (generally log BCF < 0.5). For these water-soluble dyes, log P (log K_{OW}) showed a linear relationship with log BCF; thus, it was expected that dyestuffs with higher K_{OW} would bio-accumulate. Nonetheless, water-insoluble organic pigments with extremely high partition coefficients did not bio-accumulate probably due to their extremely low water and fat solubility and also the BCF values for disperse dyes, i.e. scarcely soluble compounds with a moderately lipophilic nature, were much lower than expected. In all cases, log BCF < 2,

which indicates that none of the dyes tested showed any substantial bio-accumulation (Anliker 1979; Anliker et al. 1981, 1988; Anliker and Moser 1987; Van der Zee 2002).

2.2 Eco-toxicity of dyestuffs

The eco-toxicological effects of several distinct dyes have been intensively investigated during the last decades. These toxicity studies diverge from tests with aquatic organisms (fish, algae, bacteria, etc.) to tests with mammals. Furthermore, research has also documented the inhibitory effects of dyes on the activity of both aerobic and anaerobic bacteria in wastewater treatment consortia. The acute toxicity of dyestuffs is generally low. Algal growth (photosynthesis), tested with 56 and 46 commercially dyestuffs, respectively, was generally not inhibited at dye concentrations below 1 mg/L and with cationic–basic dyes being the most acutely toxic dyes for algae (Greene and Baughman 1996; Little and Chillingworth 1974). Fish mortality tests showed that 2% out of 3,000 commercial dyestuffs tested had LC_{50} (lethal concentration causing 50% of mortality) values below 1 mg/L. The most acutely toxic dyes for fish are basic dyes, especially those with a tri-phenylmethane structure. Fish also seem to be relatively sensitive to many acid dyes (Clarke and Anliker 1980). Mortality tests with rats evidenced that only 1% out of 4,461 commercial dyestuffs tested had LC_{50} values below 250 mg/kg body weight (Clarke and Anliker 1980). Therefore, acute sensitisation reactions by humans to dyestuffs often occur. Especially, some disperse dyes have been found to cause allergic reactions, i.e. eczema or contact dermatitis (Specht and Platzek 1995).

Chronic effects of dyes have been studied for several decades with special emphasis on food colorants, usually azo compounds. Furthermore, the effects of occupational exposure to dyestuffs of human workers in dye manufacturing and dye utilising industries have received attention. Azo dyes in purified form are hardly ever directly mutagenic or carcinogenic, except for some azo dyes with free amino groups in their structure. However, several aromatic amines readily produced by reductive azo cleavage are known as mutagens and carcinogens (Van der Zee 2002).

3 Biological wastewater treatment systems

The biodegradation of azo dyes has been reported for a wide variety of microorganisms including fungi, yeasts and bacteria both in pure and mixed cultures (Stolz 2001). However, successful applications (e.g. at full-scale) have only been reported for wastewater treatment systems combining bacterial consortia under anaerobic and aerobic conditions. Several research papers and full-scale applications have suggested that combined anaerobic–aerobic biological treatment systems are the most suitable technologies available for the treatment of textile effluents, based on chemical oxygen demand (COD) and colour removal efficiencies and on operational costs (Van der Zee and Villaverde 2005). For instance, application of upflow anaerobic sludge bed (UASB) reactors combined with aerobic tanks yielded 95–100% of decolourisation and achieved 95–98% COD removal efficiencies during the treatment of textile effluents at full scale (Jianrong et al. 1994; Kuai et al. 1998). In general, the anaerobic step is considered as the rate-limiting step during the biodegradation of azo dyes mainly due to electrons transfer restrictions (e.g. very slow transfer of electrons to azo bounds) and to toxicity over the bacteria responsible for reducing azo dyes in anaerobic treatment systems (Dos Santos et al. 2007).

4 Anaerobic reduction of azo dyes

The reductive decolourisation of azo dyes comprises the cleavage of the dyes' azo linkages, resulting in the formation of–generally colourless, but potentially hazardous–aromatic amines. The first study related to the reduction of an azo compound by bacteria was reported by Brohm and Frohwein (1937), who documented the reduction of azo dyes used in food-processing industries by lactic acid bacteria isolated from human intestine. The study was relevant due to the potential exposure, which represents the formation and eventual bioaccumulation of carcinogenic aromatic amines, derived from the dyes' cleavage, in humans. Since then, multiple research studies have documented the capacity of a large diversity of bacteria and archaea to reduce azo dyes under anaerobic conditions (Stolz 2001; Dos Santos et al. 2007). In the following sections, the mechanisms

involved in the reductive decolourisation of azo dyes and the role of different bacterial groups in azo dyes reduction are described.

4.1 Mechanisms for the reduction of azo dyes under anaerobic conditions

Different biochemical mechanisms might be involved in the reductive decolourisation of azo dyes depending on the type of microorganism and on the environmental conditions prevailing. Several redox active compounds commonly found in a wide variety of bacteria (e.g., reduced flavins and hydroquinones) are capable of rapidly transferring reducing equivalents to azo dyes causing their reductive decolourisation (Stolz 2001). Figure 1 shows the mechanisms involved in the reductive decolourisation of an azo dye mediated by a redox mediator (RM). The spontaneous reaction of the reduced form of different RM with azo dyes allows for very unspecific reductive processes. The capacity of an electron shuttling compound to be effective as a RM for azo dye reduction theoretically depends on its standard redox potential (E_0'), which should ideally be in between those of the two eventual half reactions, the reduction of an azo dye and the oxidation of a primary electron donor:

– A mediator's E_0' should not be much lower (ideally higher or less negative) than that of the biological reducing system (typically −320 mV, representing the E_0' of nicotinamide-phosphate (NAD(P)H), the cofactor with the lowest oxidation–reduction potential) or the bulk reductant (e.g. −270 mV in case of sulphide) –otherwise it will not be reduced sufficiently. Hence the observed failure of cyanocobalamin (Dos Santos et al. 2004) and ethyl viologen (Kudlich et al. 1997) to act as a mediator is most probably due to their too low E_0' values: −530 and −480 mV, respectively. Methyl viologen ($E_0' = -440$ mV) was the most reduced shuttling compound with clear ability to act as a RM for azo dye reduction. It modestly increased the rate of Acid Red 27 reduction by *Sphingomonas* sp. strain BN6 (Kudlich et al. 1997) but its impact on the reduction of Acid Yellow 23 by *Bacteroides thetaiotaomicron* was huge, a 4.5-fold increase of the reaction rate, similar or even better than that of mediators with a higher E_0' (Chung et al. 1978). Especially the latter result is remarkable, since methyl viologen's E_0' is considerably lower than the indicated boundary level of −320 mV.
– Likewise, a mediator's E_0' should not be much higher (ideally lower or more negative) than that of the azo dye –otherwise it will not reduce the azo linkage at sufficient rates. Nevertheless, the observed failure of 1,4-benzoquinone to act as a RM (Dos Santos et al. 2005b) and the poor impact

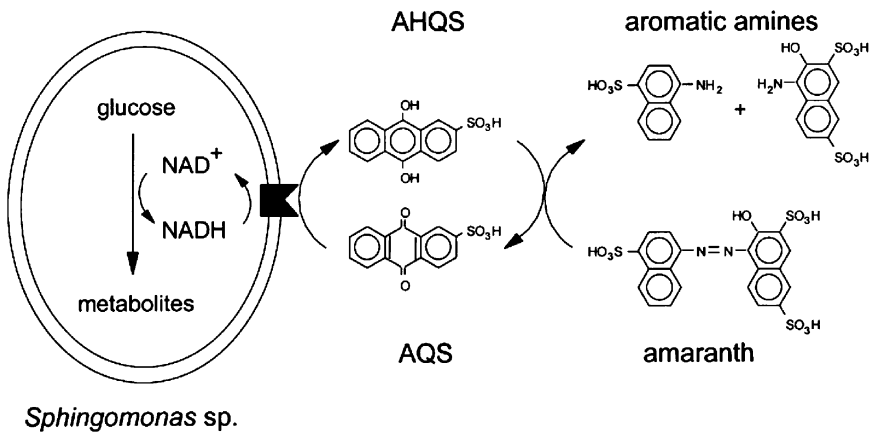

Fig. 1 Proposed mechanism of anthraquinone-2-sulphonate (AQS) mediation of amaranth dye decolourisation by *Sphingomonas* sp. Strain BN6. AQS is reduced by the membrane bound respiratory chain enzyme, NADH:ubiquinone oxidoreductase (*filled box*) to anthrahydroquinone-2-sulphonate (AHQS) and AHQS transfers the electrons to the dye, causing azo cleavage (Kudlich et al., 1997)

reported for menadione (Chung et al. 1978; Albuquerque et al. 2005) are most probably due to their too high E_0'-values: +263 and −19 mV, respectively.

In the E_0' range between these boundaries, there will be two opposing trends with respect to a mediator's E_0' and its impact on the rate of azo dye reduction. In the second step of mediated azo dye reduction, i.e. the chemical reaction between the reduced RM and the azo dye, the reaction rate will increase with decreasing E_0' of the mediator, as has been demonstrated with the reduction of Acid Red 27 by a series of chemically reduced quinones with different E_0' values (Rau et al. 2002). In the first step of mediated azo dye reduction, i.e. reduction of the mediator, an opposite trend, an increase of the reaction rate with increasing E_0' of the mediator, can be expected. The rate-limiting step of mediated azo dye reduction will thus be influenced by the mediator's E_0', with either the reduction of the dye or that of the mediator being rate-limiting, at higher and lower E_0' values, respectively. At midrange, combination of both trends may reveal the optimum mediator's E_0' (unique for each dye/reducing system combination), where both reaction steps proceed at equal rate. However, in biological systems, such an optimum E_0' could not be confirmed: studies with multiple redox mediators under otherwise similar conditions did not show any relationship between a mediator's E_0' and the rate of azo dye reduction (Chung et al. 1978; Kudlich et al. 1997; Rau et al. 2002), and mediators with identical E_0' (flavin-adenine dinucleotide (FAD), flavin mononucleotide (FMN) and anthraquinone-2-sulphonate (AQS)) often had largely different impacts. For instance, Kudlich et al. (1997) observed that Acid Red 27 reduction by *Sphingomonas* sp. strain BN6 was stimulated to a decreasing extent by the following mediators (values between brackets show E_0' in mV): AQS(−218) > lawsone(−139) > anthraquinone-2,6-disulphonate (AQDS) (−184) > benzyl viologen(−360) > methyl viologen(−440) > FAD (−219). Moreover, the impact of five quinoid mediators on the reduction of Acid Red 27 by ten pure cultures of distinct classes of bacteria showed large differences between cultures, with different sequences of these five compounds when ranged according to their effect (Rau et al. 2002). These results reflect the diverging and apparently E_0'-independent preferences of biological mediator reduction. In contrast, in chemical systems, the mediator's E_0' may very well be more important in determining its reduction rate. Therefore, mediated azo dye reduction, at least in biological systems, is largely independent of the mediator's E_0', and that this independence is probably mostly due to reduction of the RM rather than to that of the dye.

A property of electron shuttling compounds that deserves attention is the ability to cross cell membranes. It has been demonstrated that the azoreductase activity of cell extracts can be much higher than that of intact cells and that the cell membrane forms a barrier for dyes and mediators (Kudlich et al. 1997; Russ et al. 2000). Hence, mediated azo dye reduction by *Sphingomonas xenophaga*, with the sulfonated quinone AQS as the optimum mediator, is presumably associated with the membrane-bound respiratory chain enzyme NADH:ubiquinone oxidoreductase (Kudlich et al. 1997; Rau et al. 2002), and several other species are likely to use similar membrane-associated enzyme systems. However, as has recently been shown for *E. coli*, the mechanism can also be based on intracellular enzymes in combination with a mediator that diffuses through the cell membrane. *E. coli*'s mediator-dependent 'azoreductase' was identified as two cytosolic oxygen-insensitive nitroreductases, acting as quinone reductases. The mediator was lawsone, the only quinone with a mediator-feasible E_0' that could be reduced by the enzymes (Rau and Stolz 2003). Lawsone's apparent ability to cross the cell membrane is probably related to its small size and its relative lipophilicity (no charged substituents). Depending on the preferences of intracellular reductases in other species, there may be more shuttling compounds with membrane-crossing properties that could act as powerful mediators for azo dye reduction.

More recently, azo dyes could be demonstrated as terminal electron acceptors during the anaerobic oxidation of organic substrates by *Shewanella decolorationis* S12. The biochemical mechanism of azo respiration involves a formate dehydrogenase, a hydrogenase, cytochrome P450, menaquinone and an azoreductase located at the external site of the cellular membrane (Hong et al. 2007). Figure 2 illustrates the mechanisms involved in azo respiration by *S. decolorationis* S12.

Further studies were conducted to elucidate electron transfer pathways for azo reduction by

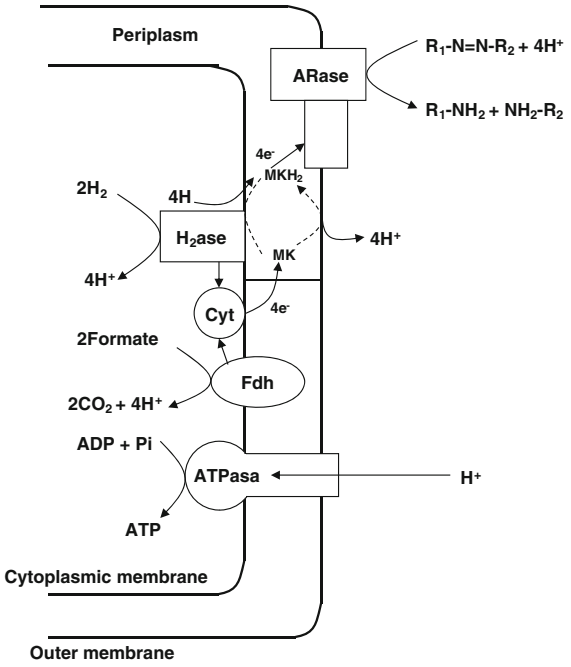

Fig. 2 Biochemical pathway proposed for the azorespiration electron transport chain in *Shewanella decolorationis* S12. *H2ase* hydrogenase, *Fdh* formate dehydrogenase, *cyt* cytochrome P450; MKH2, hydroquinone of MK; *MK* menaquinone, *Arase* azoreductase (Hong et al. 2007)

S. decolorationis S12 using a mutant S12–22 which had a transposon insertion in ccmA. The results suggested that there are two different pathways for electron transport to azo bonds. Mature c-type cytochromes were identified as essential electron mediators for the extracelular reduction of azo dyes by intact cells, while an alternative pathway without the involvement of mature c-type cytochromes, NADH-dependent oxidoreductase-mediated electron transfer pathway could reduce lowly polar sulfonated azo dyes inside the whole cells or highly polar sulfonated azo dyes in the cell extracts without bacterial membrane barriers (Chen et al. 2010).

4.2 Role of different bacterial groups in azo dyes reduction

4.2.1 Fermentative and methanogenic microorganisms

As mentioned above, the first studies documenting the bacterial reduction of azo dyes was reported on lactic acid bacteria by Brohm and Frohwein (1937). More recently, several different fermentative bacteria, commonly found in the anaerobic consortia of anaerobic wastewater treatment systems, have been reported to be capable of reducing azo dyes (Table 2). The rate and extent of reduction of azo dyes depends on the fermentative microorganism, the type of dye and the initial concentration. It is important to mention that hydrolytic and fermentative bacteria may play an important role during the anaerobic treatment of textile wastewaters because this kind of effluents contain different polymers, such as starch, which are utilized during the production of fabrics (Dos Santos et al. 2006a). In fact, high decolourisation efficiencies have been achieved in anaerobic bioreactors in which starch and carboxymethyl cellulose have been used as energy source (Isik and Sponza 2008; Gnanapragasam et al. 2010).

Recent experiments documented in detail the role of glucose-fermenting bacteria on the reductive decolourisation of azo dyes. Glucose is a relevant substrate because it derives from the hydrolysis of polymers, such as starch (Dos Santos et al. 2006a). For that purpose, sludge incubations were amended with selective inhibitors of fermentative (vancomycin) and methanogenic (2-bromoethane sulfonate (BES)) microorganisms during the reductive decolourisation of azo dyes (Dos Santos et al. 2005a, 2006b). Poor decolourisation of Reactive Red 2 (RR2) and Reactive Orange 14 (RO14) occurred in sludge incubations inhibited by vancomycin, as compared with uninhibited controls, suggesting the important contribution of glucose-fermenting bacteria. Furthermore, sludge incubations inhibited by BES (e.g. no methane production detected) showed a higher decolourisation rate compared to uninhibited controls, confirming the contribution of glucose-fermenting microorganisms. These results also suggested that methanogens might not be involved in the reduction of azo dyes; however, as discussed below, methanogenic microorganisms might have used an alternative pathway for reducing azo dyes in which methyl coenzyme M is not involved.

The specific role of methanogenic archaea in the reductive decolourisation of azo dyes has not been fully elucidated, although it is conceivable that they may play a major role considering that high decolourisation efficiencies have been related with high biogas productions in anaerobic bioreactors (Cervantes et al. 2001; Van der Zee et al. 2001; Dos Santos

Table 2 Examples of facultative, microaerophilic and strictly anaerobic bacterial cultures, which are able to decolourise azo dyes under anaerobic conditions

Organism	Dyes	Activity	Decolourisation (%)	Comments	Reference
Clostridium perfringens ATCC 3626		μ mol/mL/h			
	Amaranth (16185)	0.74	–	Dye concentration of 0.033 mM.	Semdé et al. 1998
	Methyl Orange (13025)	0.62	–		
	Orange II (15510)	0.70	–		
	Tartrazine (19140)	0.67	–		
Bacteroides fragilis		μ mol/mL/h			
	Amaranth (16185)	0.30	66.0	After 6 h of incubation. Dye concentration of 0.1 mM.	Bragger et al. 1997
	Orange II (15510)	0.20	37.0		
	Tartrazine (19140)	0.08	9.0		
Pseudomonas GM3	Acid Violet 7 (18055)	–	97.4	After 72 h of incubation. Dye concentration of 100 mg L^{-1}	Yu et al. 2001
	Reactive Blue 2 (61211)	–	18.3		
	Acid Green 27 (61580)	–	75.6		
	Acid Red 183 (18800)	–	20.1		
	Indigo Carmine (73015)	–	69.0		
Sphingomonas xenophaga BN6		μ mol/min/g protein			
	Acid Red 27 (16185)	0.10	–	Dye concentration of 0.1 mM.	Rau et al. 2002
	Acid Orange 20 (14600)	0.10	–		
	Acid Orange 7 (15510)	0.30	–		
	Acid Red 14 (14720)	0.20	–		
	Acid Yellow 23 (19140)	0.10	–		
	Acid Black 1 (20470)	0.30	–		
Enterococcus faecalis		AU $\times$ 10^{-2}/mg protein			
	Methyl Red (13020)	1.81	99.4	After 20 h of incubation. Dye concentration of 0.2 mM	Chen et al. 2004
	Orange II (15510)	1.39	95.1		
	Orange G (16230)	1.20	64.1		
	Amaranth (16185)	1.37	99.5		
Acidaminococcus fermentans	Tartrazine (19140)	–	4.0	After 150 min of incubation. Dye concentration of 2 mM	Chung et al. 1978
	Sunset Yellow (15985)	–	9.0		
	Methyl Orange (13025)	–	66.0		
	Orange II (15510)	–	72.0		
	Amaranth (16185)	–	5.0		
	Allura Red 40 (16035)	–	0.0		

Table 2 continued

Organism	Dyes	Activity	Decolourisation (%)	Comments	Reference
Eubacterium biforme	Tartrazine (19140)	–	4.0	After 150 min of incubation. Dye concentration of 2 mM	Chung et al. 1978
	Sunset Yellow (15985)	–	22.0		
	Methyl Orange (13025)	–	79.0		
	Orange II (15510)	–	81.0		
	Amaranth (16185)	–	19.0		
	Allura Red 40 (16035)	–	11.0		
Staphylococcus arlettae VN-11	Reactive Yellow 107 (*)	–	98.5	Microaerophilic conditions. After 48 h of incubation. Dye concentration of 100 mg L^{-1}	Franciscon et al. 2009a
	Reactive Black 5 (20505)	–	100		
	Reactive Red 198 (18221)	–	97.7		
	Direct Blue 71 (34140)	–	99		
Klebsiella sp. VN-31	Reactive Yellow 107 (*)	–	100	Microaerophilic conditions. After 168 h of incubation. Dye concentration of 100 mg L^{-1}	Franciscon et al. 2009b
	Reactive Black 5 (20505)	–	94		
	Reactive Red 198 (18221)	–	98		
	Direct Blue 71 (34140)	–	94		
E. coli	Congo Red (22120)	–	98	After 9 days of incubation. Dye concentration of 100 mg L^{-1}	Işik and Sponza 2003
	Direct Black 38 (30235)	–	72		
Pseudomonas sp.	Congo Red (22120)	–	100	After 5 days of incubation. Dye concentration of 100 mg L^{-1}	Işik and Sponza 2003
	Direct Black 38 (30235)	–	83		
Shewanella decolorationis S12	Amaranth (16185)	–	100	After 31 h of incubation. Dye concentration of 2 mM	Wang et al. 2010
	Amaranth (16185)	–	100	After 44 h of incubation. Dye concentration of 6 mM	

Table 2 continued

Organism	Dyes	Activity	Decolourisation (%)	Comments	Reference
Lactobacillus casei TISTR 1500		mg/g cell/h			
	Methyl orange (13025)	6.1	–	Complete decolourisation after 3 h of incubation. Dye concentration of 0.23 mM	Seesuriyachan et al. 2007
	Tropaeoline O (14270)	5.0	–		
	Crocein orange G (15970)	6.1	–		
	Orange II (15510)	8.7	–		
	Acid red 151 (26900)	0.54	–		
	Acid orange 8 (15575)	1.1	–		
	Ponceau 3R (16155)	8.8	–		
	Methyl red (13020)	31.0	–		
	Congo red (22120)	2.4	–		
Bacillus sp. ADR		mg/g dry cell/h			
	Reactive orange 16 (17757)	2.62	88	After 24 h of incubation. Dye concentration of 100 mg L^{-1}	Telke et al. 2009
	Solvent Red 24 (26105)	–	90		
	Reactive Red 2 (18200)	–	90	After 12 h of incubation. Dye concentration of 100 mg L^{-1}	
	Acid Red 2 (13020)	–	86		
Pseudomonas sp. SUK1	Red BLI (*)	–	99.3	After 60 min of incubation. Dye concentration of 50 mg L^{-1}	Kalyani et al. 2008
	Reactive Navy blue RX (*)	–	85.3	After 80 min of incubation. Dye concentration of 50 mg L^{-1}	
	Reactive Red HE3B (25810)	–	93.5	After 360 min of incubation. Dye concentration of 50 mg L^{-1}	
Dyella ginsengisoli LA-4	Acid Red GR (27290)	–	89	After 20 h of incubation. Dye concentration of 85 mg L^{-1}	Zhao et al. 2010
Citrobacter sp. CK3	Reactive Red 180 (*)	–	95	After 36 h of incubation. Dye concentration of 200 mg L^{-1}	Wang et al. 2009

Colour index is in parenthesis

(*) not available

et al. 2005b). Recent experiments revealed the important contribution of methanogens on azo dyes reduction. Indeed, different methanogenic strains, including *Methanothermobacter thermoautotrophicus* ΔH, *Methanobacterium* NJ1 and *Methanosarcina barkeri* were able to decolourise RR2 with hydrogen as an electron donor in axenic cultures (Dos Santos et al. 2006b). However, the presence of riboflavin as a RM was essential to achieve the reductive decolourisation of RR2 by *M. thermoautotrophicus* ΔH and *Methanobacterium* NJ1, whereas *M. barkeri* was capable of reducing RR2 even in the absence of this RM. Interestingly, *Methanobacterium* NJ1 and *M. barkeri* could reduce RR2 even in the presence of the selective inhibitor of methanogens, BES, suggesting that these strains were able to decolorize RR2 by a biochemical mechanism in which methyl coenzyme M is not involved (Li et al. 2010).

Further experiments revealed negligible methane production during the course of reductive decolourisation of different azo dyes by anaerobic consortia when different methanogenic substrates (acetate, methanol, hydrogen and formate) were utilised (Dos Santos et al. 2006b; Cervantes et al. 2008). Methanogenic activity was more strongly inhibited by increasing the concentration of azo dyes, which may be due to competition between methanogenesis and azo dyes reduction for the reducing equivalents available in the cultures. This hypothesis is supported by the reversibility of inhibition observed after azo dyes were completely reduced (Dos Santos et al. 2006b; Cervantes et al. 2008). Addition of riboflavin as a RM in decolourisation assays attenuated the inhibitory effects of azo dyes by decreasing the exposure of methanogenic microorganisms to azo dyes (Cervantes et al. 2008). The application of quinoid RM to continuous decolourising bioreactors also recovered the methanogenic activity and decolourisation efficiency, which were collapsed due to toxicity effects or RR2 (Van der Zee et al. 2001).

4.2.2 *Sulphate-reducing bacteria*

Textile-processing wastewaters may contain high sulphate concentrations. Sulphate is generally an additive of dye baths or it may be formed by the oxidation of more reduced sulphur species used in dyeing processes, such as sulphide, hydrosulphide, and dithionite (Van der Zee et al. 2003). Sulphate may have different effects on the reduction of azo dyes. Firstly, sulphate may compete with the dyes, as an electron acceptor, for the electrons available, depending on the capacity of the inocula to carry out sulphate reduction and on the concentration of sulphate. Secondly, electron equivalents may be generated by the reduction of sulphate via anaerobic substrate oxidations; thus, reduced cofactors involved in sulphate reduction may also play a role in azo dye reductions. Finally, sulphide generation, via sulphate-reduction, may also contribute to the reduction of azo dyes (Van der Zee et al. 2003; Cervantes et al. 2007).

Several studies have documented the reductive decolourisation of many distinct azo dyes under sulphate-reducing conditions (Van der Zee et al. 2003; Albuquerque et al. 2005; Cervantes et al. 2006, 2007); nevertheless, scarce information is available elucidating the mechanisms involved in the reductive decolourisation of azo dyes under these conditions. The collected evidence from these studies indicates that both the biological activity linked to sulphate reduction and the chemical reduction by biogenic sulphide may be important mechanisms, which promote the reduction of azo dyes under sulphate-reducing conditions. The simultaneous reduction of azo dyes and sulphate appears as evidence of the biological contribution in several studies (Van der Zee et al. 2003; Albuquerque et al. 2005). However, the role of biogenic sulphide is not always relevant for azo dyes reduction as several azo dyes remain unaffected or are poorly reduced in chemical incubations with sulphide provided as a unique reducing agent (Cervantes et al. 2006, 2007). The application of RM, such as riboflavin, has enhanced the contribution of sulphide as an effective reducing agent for azo dyes reduction. Certainly, addition of riboflavin (20 μM) to chemical incubations provided with sulphide as an electron donor increased up to 44-fold the rate of decolourisation of different azo dyes (Cervantes et al. 2007). The quinoid RM, AQDS, also increased the rate of reduction of Acid Orange 7 (AO7) up to 100-fold (Van der Zee et al. 2000). Further studies elucidated that biogenic sulphide plays a major role during the microbial decolourisation of different azo dyes when riboflavin is available as a RM (Cervantes et al. 2007).

5 Conclusions

The information described here indicates that several distinct biochemical mechanisms are involved in the reductive decolourisation of azo dyes by bacterial communities. Moreover, several bacterial groups commonly active in anaerobic wastewater treatment systems, such as fermentative, methanogenic, sulphate-reducing and azo-reducing microorganisms, appeared to have important contribution during the reduction of azo dyes under anaerobic conditions. Nevertheless, several microbiological and biochemical aspects need to be further investigated in order to better understand the mechanisms involved in the bacterial reduction of azo dyes and to enhance the applicability of bacterial consortia for textile wastewater treatment. For instance, most studies have been conducted under ideal culture conditions and the impact of pollutants co-existing in textile effluents (e.g. surfactants, disinfectants, size agents, etc.) on the reduction of azo dyes has poorly been assessed. Moreover, the role of syntrophic interactions of different bacterial groups on azo dyes reduction needs to be addressed in order to optimize decolourising processes.

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