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Biodegradation of Textile Azo Dyes by a Bacterial Consortium From Contaminated Soil

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ABSTRACT

Azo dyes are the largest chemical class of synthetic colorants, and a good share of what is applied in textile mills leaves in the wastewater. Bacterial consortia isolated from dye-contaminated soil are often proposed as a cheap way to clean it up, and reports of rapid decolorization are common. This narrative review asks what such reports can and cannot show. It draws on published work on azo dye chemistry, the enzymes that cleave the azo bond, the aerobic fate of the aromatic amines that result, and the design of consortium studies, and it reports no new data and no local measurements. Loss of colour is best read as the first step of a longer pathway: reductive cleavage is usually fast under low-oxygen conditions, but the amines it releases can be as worrying as the dye and need a second, oxidative stage that colour readings do not track. Consortia often outperform single isolates because the members divide the work, yet that same division makes them hard to reproduce, to describe and to scale. Many reports rely on colour alone, on single dyes in clean media, and on communities that are never characterised. The review concludes that consortium treatment is plausible and sometimes impressive, that complete detoxification is far less often shown than complete decolorization, and it sets out the controls, chemistry and toxicity tests that a convincing study would contain.

Keywords: Azo dyes; Biodegradation; Bacterial consortium; Azoreductase; Aromatic amines; Textile wastewater; Narrative review

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Article Class: Narrative Review

1. Introduction

Textile dyeing is a wasteful process. Not all of the dye applied to fibre binds to it, and what remains in the bath is discharged with salts, auxiliary chemicals and alkali or

acid. Of the many chemical classes in use, azo dyes, which carry one or more –N=N– linkages, are the largest, and they dominate the colour range for cotton, wool and synthetic fibres (1, 2). Coloured effluent is



unsightly, but the worry runs deeper than appearance: dyes cut light penetration in receiving water, many are designed to resist fading and so resist breakdown, and some of their breakdown products are hazardous (3, 4).

Microbes have been studied as a gentler and cheaper alternative to physical and chemical treatment for more than three decades. Bacteria isolated from soil that has long received dye-laden water are an obvious place to look, since the organisms found there have had time to adapt to the compounds (5, 6). A recurring finding is that mixed cultures, or consortia, decolorize dyes more completely than their separate members do (7, 8). It is easy to see why the idea appeals to a laboratory with a shaking incubator and a spectrophotometer.

What is harder to see is how much a decolorization report actually shows. Loss of colour means that the chromophore, usually the azo bond, has been broken. It does not mean that the dye has been mineralised, that the products are harmless, or that the same community will work in a mill drain. This review looks at what is known about bacterial consortia from contaminated soil as agents of azo dye breakdown, at the chemistry that colour readings cannot see, and at the way such studies are designed and reported. It is a narrative review of published literature. No data from any particular soil, site or isolate are reported here, and nothing in the text should be read as a local measurement. The aim is a fair account of what colour loss does and does not tell us.

2. Literature Review

2.1 Azo Dyes and Why They Persist

Azo dyes are built around one or more azo bonds joining aromatic rings, often carrying sulfonate groups that make them water soluble and give them affinity for

fibre. The same features that make them good dyes make them poor candidates for ordinary biodegradation. They are xenobiotic, in the sense that natural compounds with this linkage are rare, and the sulfonate groups slow uptake into cells (4, 9). Under the oxygen-rich conditions of most aerobic treatment plants, the azo bond tends to survive, so conventional activated sludge removes colour mainly by sorption onto the biomass (10, 11).

Their toxicity is a mixed picture. Many intact azo dyes are of low acute toxicity, and the concern lies with what is released when the bond is broken. Reduction yields aromatic amines, some of which are mutagenic or carcinogenic, and the literature on structure and toxicity is large (12, 13). Textile effluent that reaches drinking water sources has been shown to carry mutagenic activity (14). A treatment process judged only by colour could therefore leave water that looks cleaner while being, in some respects, more hazardous. A treatment that removes colour but leaves the amines behind has moved the problem, not solved it.

The wider treatment literature offers many routes, from adsorption and membranes to oxidation and biological processes, each with its own trade-offs (1, 15). Table 1 sets out the main families and their characteristic strengths and weaknesses. Biological methods are attractive because they promise lower reagent use and, in the best case, complete destruction (16); the reviews that compare them agree that no single approach suits every dye or every effluent (17, 18).

2.2 Enzymes That Cut the Azo Bond

The first step in bacterial breakdown is usually reductive. Purified azoreductases from several organisms transfer electrons from reduced nicotinamide cofactors to the dye, splitting the azo bond and giving two amines. An early characterisation of an



Orange II azoreductase from a *Pseudomonas* strain showed how specific such enzymes can be to the dye structure (22), and a gene for an azoreductase was later cloned from a *Bacillus* isolated from soil (23). Reviews of the enzymology stress that bacterial azoreductases differ in cofactor requirement, oxygen sensitivity and substrate range (4, 24).

Table 1. Broad families of treatment for dye-bearing textile wastewater.

Approach	Main strength	Main weakness
Physical (adsorption, membranes)	Fast, no biology to maintain (1, 15)	Moves the dye to another phase
Chemical oxidation	Strong colour removal (1)	Reagent cost, by-products
Anaerobic biological	Cleaves the azo bond (11, 19)	Leaves aromatic amines
Aerobic biological	Can degrade some amines (20)	Poor on many intact dyes
Sequential anaerobic–aerobic	Addresses both steps (19, 21)	Two stages; more control needed

Note: Qualitative summary of points made in the cited reviews; practice varies between mills.

Not every reduction needs an enzyme that touches the dye directly. Cytoplasmic flavin reductases can pass electrons to low-molecular-weight carriers, and these reduced carriers then cleave the azo bond outside the cell (25). This matters for sulfonated dyes that enter cells poorly. Redox mediators formed during the breakdown of naphthalenesulfonates have been shown to speed the reduction of azo dyes by a *Sphingomonas* strain (26), and studies of where the activity sits in the cell found that the system could be membrane associated and that added redox compounds raised the rate (27). The implication is that a community might help its members, for example by supplying a

mediator that another member cannot make.

Conditions strongly affect the outcome. Many bacteria reduce azo dyes much faster when oxygen is scarce, since oxygen competes for the reduced cofactors, and a static or low-aeration culture often decolorizes where a vigorously shaken one does not (6, 28). The chemical structure of the dye also matters: substituents on the rings change the rate at which a given strain can reduce it (29). Table 2 lists the successive steps that a complete breakdown involves and how each is usually followed in the laboratory. Each of these effects is small alone, but together they decide whether a culture behaves on the bench as it did in the flask.

Table 2. Steps in bacterial azo dye breakdown and how each is usually followed.

Step	What happens	How it is followed
Uptake or contact	Dye meets cells or reduced carriers (26, 27)	Rarely measured
Azo bond cleavage	Colour lost; amines formed (23, 25)	Spectrophotometry
Amine transformation	Some amines oxidise or polymerise (30)	Chromatography, mass spectrometry
Ring opening	Aromatic rings degraded aerobically (4, 20)	Oxygen use, carbon loss
Detoxification	Harm to test organisms falls (12, 14)	Bioassays, genotoxicity tests

Note: The last column states what a reader should look for, not what every study measured.

2.3 Amines and the Aerobic Second Stage

Once the azo bond is cut, the question changes. The aromatic amines produced



are often colourless and are stable under the reducing conditions that formed them. Some oxidise or polymerise when exposed to air and can recolour the liquid, which complicates colour readings. Analytical work on these products points to chromatographic and spectroscopic methods as necessary, because simple absorbance cannot distinguish one amine from another (30). Several amines are mutagenic (13), and this is the reason that sequential treatment has attracted so much attention.

A classic demonstration of the idea is the mineralisation of a sulfonated azo dye by a bacterial consortium in which the members carried out different parts of the job: one reduced the dye, and others degraded the naphthalene amine that resulted (20). Later work paired anaerobic granular sludge with aerobic enrichment cultures selected for growth on aromatic amines (21), and reviews of bioreactor studies conclude that a combined anaerobic-aerobic scheme is the most promising biological design for many dyes (19). The weakness is practical, not conceptual: two stages need two sets of conditions, and a single flask cannot easily supply both. That is a modest claim, and it is the one the literature will currently carry.

2.4 Why Consortia, and Why from Contaminated Soil

Microbial communities can do things that single strains cannot. Different members can specialise in successive steps, share cofactors or mediators, remove inhibitory products, and tolerate variation in conditions that would stop a pure culture. The synthetic biology literature treats consortia as a way of splitting a heavy metabolic task between organisms (31), and for dye breakdown the logic is the same. A report comparing a mixed culture with its constituent isolates found the mixture more effective across several dyes than any single member (7).

Soil that has received dye-bearing water is a sensible place to look, because it concentrates organisms already exposed to the compounds, but soil is also extremely diverse and most of its organisms are hard to grow in the laboratory (5, 32). A culture that works is therefore a selected fraction of what was there, and enrichment on a dye or a rich medium will favour some organisms over others. Isolates from contaminated soil have been reported to decolorize a range of disperse and acid dyes (33), and mixed cultures from soil, sludge or dye-contaminated sites have been described for reactive and other dyes (8, 34). This is why the same strain can look excellent in one laboratory and ordinary in the next.

Living together carries costs as well. Bacteria in mixed communities compete for resources and can inhibit one another (35), and many dye-degrading cells live in biofilms whose matrix changes diffusion, protection and the exchange of products (36). A consortium is therefore not a bag of useful parts. Its members can drift in proportion over repeated transfers, and a mixture that worked in the first month may behave differently after several subcultures. A community that has had time to settle behaves differently from one that has just been assembled.

3. Materials and Methods

3.1 Scope and Question

The review asks one question in three parts. Is it plausible that a bacterial consortium taken from dye-contaminated soil can decolorize azo dyes? Is there good reason to expect it to degrade or detoxify the products? And how far do published designs allow the answers to be generalised? The title concerns a particular kind of study, but the review reports no experiment of its own and no data from any site; it addresses the topic through the



published literature. None of these questions is hard, but each is easy to leave out.

3.2 Literature Assembly

The review draws on peer-reviewed articles in five areas: azo dye chemistry and toxicity; azoreductases and electron transfer; combined anaerobic and aerobic treatment; consortium and community ecology; and the molecular methods used to describe communities. Sources were chosen for relevance, standing in the field and clarity of method. Work on single strains is included for what it shows about mechanism, not as evidence for the performance of a mixed culture. All sources were published in or before the year of this issue.

We did not carry out a formal systematic search and do not claim to have done one. This is a narrative synthesis, and the choice of sources reflects the authors' judgement. It is a better guide to what a convincing study should contain than to the size of any reported rate, so no decolorization percentages are quoted.

3.3 Appraisal Framework

To judge how far a report can be trusted we asked five questions of it. Was loss of colour separated from adsorption, using killed-cell or sterile controls (10)? Was the community described, and by what method (37, 38)? Were the products identified (30)? Was toxicity compared before and after treatment (14)? And was the dye tested in conditions resembling real effluent, with salts and mixed dyes present (15)? Table 3 turns these questions into a short checklist. It is a reading aid and not a validated scoring tool, and every item on it can be answered from the text of a published report. It is a habit worth keeping even when the outcome is expected.

Table 3. Questions to ask of a consortium decolorization report.

Question	Why it matters	Good practice
Was a sterile or killed-cell control run?	Adsorption can mimic degradation (10)	Report both controls
Was the community described?	Cannot be reproduced otherwise (37, 39)	Name members and method
Were products identified?	Amines may remain (13, 30)	Analyse metabolites
Was toxicity tested?	Colour loss is not safety (14)	Compare before and after
Was real effluent used?	Salts and mixtures inhibit (15, 29)	Test beyond clean media

Note: Drawn from the methodological points discussed in the text; not a validated scoring instrument.

4. Results

4.1 What the Reports Show About Colour

The literature on consortia from soil and sludge is encouraging as far as colour is concerned. Mixed cultures and single isolates alike are reported to decolorize a wide range of structurally different azo dyes, often under low-oxygen or static conditions (6, 8, 33). Studies that compare a mixture with its parts often, though not always, find the mixture ahead (7), and kinetic work on one well-studied *Pseudomonas* species shows that the rate depends on the dye, the carbon source and the conditions in ways that no single number can summarise (28, 29).

Reading across these reports, the common design is a batch culture in a defined or semi-defined medium, one dye at a time, with colour followed by absorbance at the visible maximum of the dye. That design is quick, cheap and informative about the first step. It tells us much less about the conditions of a real effluent, which carries



a mixture of dyes, high salt, variable pH and fluctuating load (15, 17). Figure 1 sets the two-stage pathway beside the checks that belong at each stage.

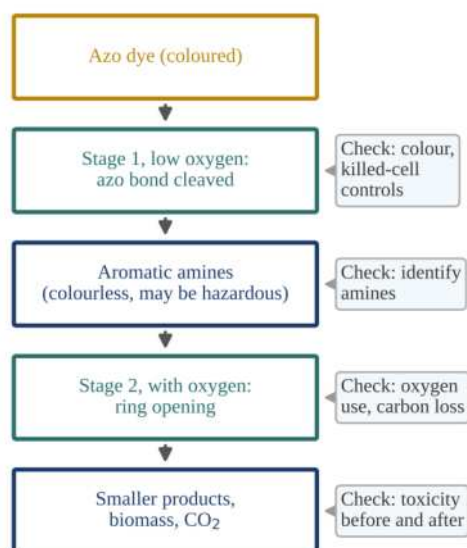


Figure 1. Two-stage pathway for azo dye treatment and the checks that belong at each stage (schematic, not data).

4.2 What Happens Afterwards

Fewer reports go beyond colour. Where they do, the picture is more mixed. Identification of metabolites from methyl red breakdown by a *Sphingomonas* strain, together with a comparison of toxicity before and after, shows that the fully worked case is possible (40). Work on a *Pseudomonas* isolate combined decolorization with toxicity tests on the products and reported lower toxicity afterwards (34). These are valuable because they tackle the second half of the question, but they are a minority, and each concerns a particular dye and strain. A reader of a decolorization paper wants one thing settled: was the dye destroyed, or only hidden?

The cases in which a complete route has been demonstrated tend to involve a division of labour. The mineralisation of a sulfonated azo dye by a consortium (20) and the coupling of anaerobic sludge with

amine-degrading enrichments (21) both work because the members do different things. In that sense, the best evidence for the value of consortia is not that they bleach faster, but that they can finish a job that single organisms leave half done. Figure 2 sketches, qualitatively, the roles that different members may play.

	Reduce azo bond	Supply mediators	Degrade amines	Form biofilm
Member A	role			role
Member B	role	role		
Member C			role	role

Schematic of possible division of labour, not data

Figure 2. Qualitative matrix of roles that different members of a consortium may play (schematic, not data).

4.3 Reproducibility and Description

A consortium is only as useful as its description. Many reports identify a few isolates by their 16S ribosomal gene (38), some follow the community by gel-based profiling (37), and high-throughput sequencing of the same gene has made much deeper description cheap and routine (39). Yet a large share of reports give no description at all, or describe only the isolates that grew on plates, which may not be the members that did the work (32). A culture that cannot be described cannot be recreated, and a result that cannot be recreated is hard to build on, and a mixture whose balance drifts over repeated transfers may not stay the same community (35, 36). Simply naming the genera present is a start, not a description of function. The point is not that such a study is poor, only that its result travels less far than its abstract suggests.



5. Discussion

5.1 Interpretation

The balance of the literature supports a cautious reading. Consortia from contaminated soil are a plausible and sometimes impressive route to azo dye decolorization, mainly because the first step is a robust, widespread capability that depends little on specific genes. What is less settled is the next step. The aromatic amines that result are the substance of the toxicity concern (13, 14), and only some studies show their fate. Colour is easy to measure and satisfying to plot, and for that reason it has come to stand in for success.

The second lesson concerns design. Interpretation of a batch decolorization test is strongest when sterile and killed-cell controls are included, the community is described, and products and toxicity are followed (10, 30). When these are missing, a report can still be useful, but its conclusions should be limited to what the data support: that colour was lost under defined conditions. The reviews that weigh bioreactor studies point to the same distinction between laboratory decolorization and demonstrated treatment (19, 41). Figure 3 summarises this reading as a short decision tree. Reviews are more useful when they say where the evidence stops than when they smooth it over.

5.2 Limitations

The review has its own limits. It is a narrative synthesis with no formal search, so relevant studies will have been missed, and the choice of sources reflects judgement. It reports no data and no site-specific measurements, so it cannot say how a particular soil consortium would perform. Because the literature is dominated by laboratory batch studies with single dyes, the review inherits the same bias: it can say more about the first step than about the full pathway, and more

about decolorization than about toxicity. Seen this way, a failure to decolorize in a short test is not proof that a community cannot do so.

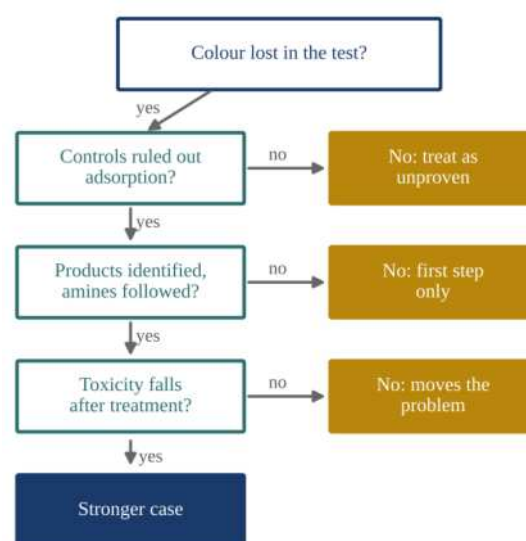


Figure 3. Decision tree for reading a report of consortium decolorization (schematic, not data).

5.3 Future Work

A convincing study of a soil consortium would do five things. It would describe the community at the start and after repeated transfer, using current sequencing methods (39). It would include sterile and killed-cell controls (10). It would identify the products of the first and second stages and state what happens to the amines (20, 30). It would test toxicity with at least one bioassay and one genotoxicity test (12, 14). And it would challenge the community with a realistic effluent, not only a clean medium. Studies that do these things would settle many of the questions this review can only frame, and would let a reader judge whether a consortium is a sound option for a treatment plant or a good laboratory story.

6. Conclusion

Bacterial consortia from dye-contaminated soil can remove the colour of many azo dyes, and the evidence for this is broad.



The evidence that they detoxify those dyes, or that they do so in real effluent, is thinner and more scattered. What the literature does show is where the benefit of a consortium lies, why a second oxidative stage is usually needed, and which controls and measurements separate a sound study from a suggestive one. Readers should treat claims of complete removal with caution until they meet those standards.

For those planning treatment, the message is practical, not discouraging. Consortia are worth pursuing, provided the community is described, the products are identified, toxicity is measured and the test conditions resemble the effluent. For researchers, the opportunity lies in carefully controlled, fully reported studies that carry a soil-derived community from a bleached flask to a measured, safer effluent. These points apply to any microbial treatment of an industrial effluent, not only to azo dyes.

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9. Conflict of Interest

The authors declare no conflict of interest.

10. Author Contributions

L.W. conceived the review and wrote the sections on dye chemistry and consortium ecology. M.F. wrote the sections on enzymes and degradation pathways. S.P. wrote the sections on toxicity and study design. All authors revised the manuscript and approved the final version.

11. Ethics Statement

This is a review of published literature. No human or animal subjects were involved and no ethical approval was required.

12. Data Availability

No new data were generated. All sources are cited in the reference list.

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