

**Extended summary of the doctoral dissertation by Kamran Ayaz entitled: *Application of Pure and Mixed Cultures of Bacteria Strains in Synthetic Dyes Removal Processes – Mechanisms and Optimisation of Process Conditions***

Synthetic dyes are produced and used on a very large scale, especially in the textile industry, because they provide high color intensity, a wide range of shades, and appropriate color fastness. Dyeing of fibers as well as other materials is carried out under strictly controlled parameters such as pH, salinity level, temperature, and the use of auxiliary agents. These conditions keep the dye stable and soluble, help it bind to the surface of fibers, and to target sites on the surface of materials other than fibers. In many conventional dyeing processes, a large part of the applied dye does not bind sufficiently or permanently to the fiber surface. As a result, significant amounts of unbound dyes enter industrial wastewater; moreover, dyes that are not fixed strongly enough on the fiber are washed out during the washing step and then also enter wastewater. Wastewater from the dyeing industry is chemically complex and, due to processed dye losses, is strongly colored. The effluent often contains not only unfixed dyes, but also salts, bases or acids, additives such as surfactants, dispersants, wetting agents, and other auxiliaries. As a result, textile wastewater is usually characterized by strong color, high organic load, variable pH, and high conductivity. These characteristics make textile effluents difficult to treat in standard wastewater treatment systems. They also explain why many laboratory results, generated under simple and clean conditions, do not always transfer well to real industrial wastewater.

The problem becomes more severe when the dyes are highly water soluble and anionic. These dyes are purposely designed to remain soluble and stable in water dye baths, to maintain color strength, and to resist fading during use. The same properties that make them effective in dyeing also make it difficult to remove from water. High solubility and strong ionic charge reduce removal efficiency by simple solid and liquid separation. In many cases, they also reduce adsorption on common carriers or biomass, unless special surface materials or high chemical doses are used. At the same time, their stable structure makes them resistant to light-driven breakdown and microbial degradation. Because of these characteristics, highly soluble anionic dyes can persist in natural waters and remain visible even at very low concentrations. Even small amounts of dye can give a very strong color to the water, which reduces visual quality and lowers light penetration. Lower light penetration can reduce photosynthesis in algae and aquatic plants. This can then disturb primary production and influence food webs and overall ecosystem function.

Among synthetic dyes, azo dyes are the most common used dye family in coloring industries. They are widely used because they can be produced in wide range of colors and structures at low cost, and they can be designed for different fibers and application classes. However, azo dyes are often difficult to treat biologically, and they create a major interpretation issue during treatment studies. In many biological systems, the azo bond, which is the N=N group, can be reduced. This reduction can quickly break the chromophore and lead to rapid color loss. If we keep color removal is the main monitoring point, then this can look like successful treatment. However, cleavage of the azo bond can also form aromatic by-products that may be colorless, persist and may need further oxidation to reduce environmental risk. In other words, a process can remove color quickly but still leave transformation products that matter for pollution control. Laboratory and field studies show that dyes can impair growth, development, and reproduction of aquatic organisms. They can also cause multi-organ injury through oxidative stress, depending on dye structure, concentration, and exposure time. Azo dyes and their transformation products are linked with their toxicity, genotoxicity and broader environmental health risks, supporting precautionary management and robust treatment before discharge in water streams.

### **Dissertation objectives and thesis statement**

This dissertation fits into this problem area through research on optimizing processes for removing synthetic dyes using pure and mixed bacterial cultures. In the first stage, research was carried out using dyes from the triphenylmethane and azo groups. However, most experiments were based on processes for removing the diazo dye EB. This dye was chosen as a test dye for several reasons described below. EB represents the group of the most used anionic dyes: strongly sulfonated, very well soluble in water, intensely colored, and particularly difficult to remove from wastewater. As an azo dye, it is designed to remain stable and soluble in aqueous dye baths. High solubility and negative charges reduce adsorption on many typical carriers, so physical removal alone is often limited unless special conditions are applied. The aromatic and sulfonated structure of EB is designed for stability, which is often associated with persistence in the environment and slow degradation when conditions are not controlled. These properties make EB a demanding test compound both for biological and bioelectrochemical treatment.

In wastewater treatment studies, color removal is often reported as the main indicator of success. However, for sulfonated azo dyes such as EB, color changes can occur without full

transformation. Chromophore behavior may change without complete breakdown, and wastewater components may influence spectra; therefore, decolorization measured by UV–Vis spectrophotometry alone is not sufficient proof of treatment. In this dissertation, UV–Vis is used to track kinetics, but conclusions are strengthened by additional analytical methods that confirm loss of the parent dye and identify transformation products. In this way, decolorization is treated as an important signal, but it is supported by stronger evidence confirming an actual chemical change resulting from biotransformation of the compound.

### **Objectives**

The main objective was to develop and validate biological and bioelectrochemical strategies that speed EB removal with pure and mixed cultures while demonstrating true chemical transformation and defining practical design limits. This was achieved through four connected experimental chapters:

1. Build a biological starting point by screening actinomycetes for decolorization of EB and other model dyes and evaluate low-cost carriers, while separating adsorption from biodecolorization.
2. Move to a controlled, optimization-driven pure culture framework using *Shewanella oneidensis* MR-1 and Response Surface Methodology (RSM), supported by analytical verification beyond UV–Vis.
3. Translate the process to a reactor-based bioelectrochemical platform using a mixed community in a double-chamber microbial fuel cell (DCMFC) coupled with aerobic post-treatment, adding electrochemical and microbial community evidence.
4. Intensify performance in a simpler single-chamber microbial fuel cell (SCMFC) by engineering the anode biointerface (PANI + glucose + gelatin concept) to improve adhesion, electron transfer, and tolerance to higher EB loads.

### **Thesis statement**

The dissertation provides fast and reliable treatment of a highly soluble sulfonated azo dye when microbial capability, a defined operating window, and microbe–electrode interface design are treated as one connected engineering problem, and when treatment is verified using chemical and mechanistic evidence rather than UV–Vis color loss alone.

### **Description of the research subject and general methods**

The research was carried out in stages, and each stage inspired the direction of research in the next stage. In the early stages, the work focuses on controlled biological systems and then moves to designed bioelectrochemical systems. At each stage, the goal is not only to improve performance but also to improve interpretation and process control. The first research stage identifies the potential of microorganisms and shows interpretative and process limitations of immobilizing biomass on natural solid carriers. In the next stage, the research was based on a pure, identified, and well-described bacterial strain, with statistical optimization carried out to determine stable process windows instead of relying on the effect of single factors. Subsequently, the research was directed toward bioelectrochemical treatment processes with mixed communities, where redox control and electrochemical monitoring provided additional control tools. The dissertation focused on the microorganism–electrode interface as a key bottleneck. By using anode interface engineering to improve early adhesion, electron transfer, and tolerance to higher dye loads, the work moved toward a simpler and more controllable treatment system.

In the first experimental chapter, the work focused on screening aimed at obtaining bacteria from the Actinomycetes group (actinomycetes) which is useful for removing synthetic dyes. The tests used the azo dye EB and two triphenylmethane dyes, Brilliant Green (BG) and Crystal Violet (CrV). Screening on solid medium is a practical way to identify isolates that can grow in the presence of dyes and form visible decolorization zones. This step is useful because it provides potential strains and shows that the microorganism resource in each sample includes organisms capable of transforming different dye structures. At the same time, this type of screening is only a preliminary selection tool. It cannot fully separate true biological transformation from physical effects. A clear zone around a colony may reflect enzyme activity, but it may also depend on dye diffusion in solid medium, local pH changes, redox changes, and sorption by biomass, for example due to dye binding by extracellular polymers. For this reason, this chapter emphasizes the need to later test promising strains in controlled liquid systems with appropriate blanks and verification controls.

An important part of the first chapter was testing cheap, natural solid carriers to stabilize biomass growth and improve process efficiency. Biomass immobilization systems are widely used in wastewater biotechnology because they increase biomass retention, reduce washout, and buffer shock loads. They also support slow-growing degraders and enable longer contact

without requiring very long hydraulic retention times. In dye treatment, immobilization may be helpful because dyes can inhibit the development of microorganisms and slow metabolism, and immobilization can create a more protective microenvironment. However, carrier systems also create an interpretative problem because carriers can participate in physical sorption of dyes. Therefore, when an immobilized-biomass system shows color removal, it is important to determine what fraction results from sorption on the carrier and what fraction results from biological conversion carried out by microorganisms.

In this dissertation, three carriers were tested: conifer wood shavings, straw, and deciduous wood chips. Only straw supported visible microbial growth during preliminary tests, so it was selected for the main carrier experiments. After 14 days, straw alone and biomass alone gave lower dye removal results, whereas straw with biomass gave higher removal results. This suggests that the carrier improved performance, through stabilizing biomass, improving retention, and increasing contact between cells and dye molecules. Under these conditions, strain 1K1 removed 66.6% of BG and 80% of EB, while strain EGK2 removed 97.8% of BG and 73% of EB (Figures. 1 and 2). These results show that actinomycetes can contribute to dye removal and that cheap carriers can improve process performance. At the same time, the results clearly indicate interpretative and process limitations. The time needed to achieve a high level of dye removal is long, about 14 days, and the observed reductions in color may reflect both physical and biological mechanisms.

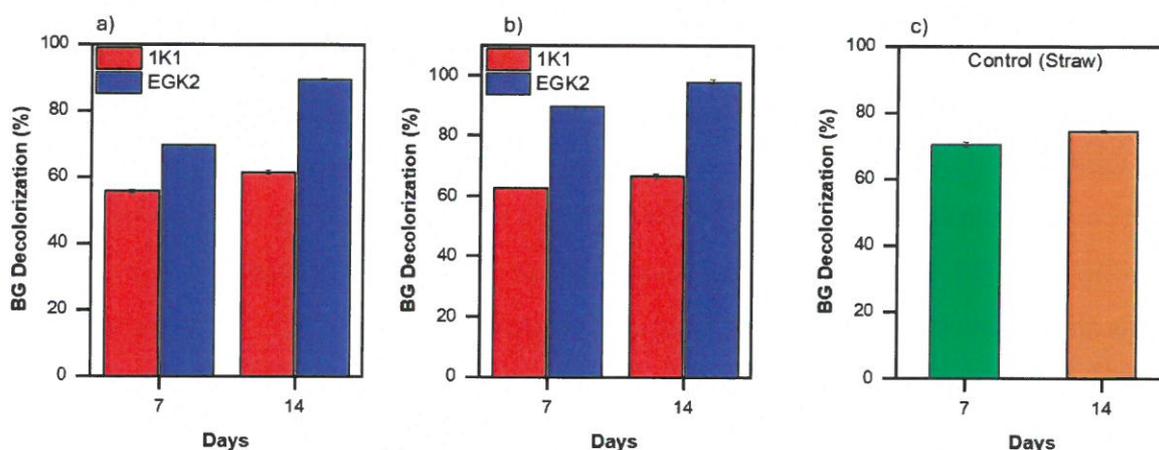


Figure 1. Percentage Removal of triphenylmethane brilliant green after 7- and 14-days a) Substrate + biomass, b) Substrate + straw + biomass, c) Straw alone

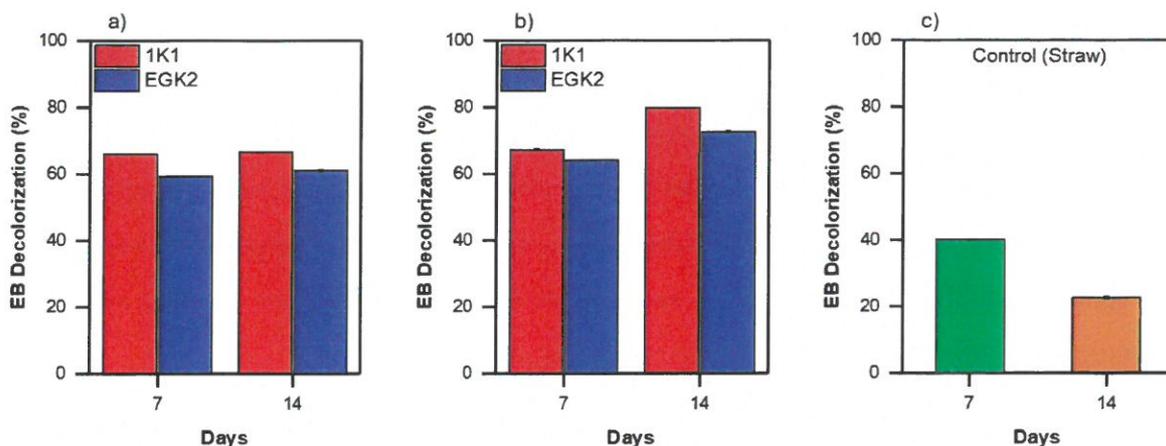


Figure 2. Percentage removal of azo dye Evans blue after 7 and 14 days A) Substrate + biomass, B) Substrate + straw + biomass, C) Straw alone

Because the dissertation focuses on mechanisms and optimization of process conditions, this stage served as a reference point and justified the need to move to the next stage, with a different direction, in which a model organism was used, and statistical optimization was performed to improve treatment speed and evidence quality.

The second experimental chapter addresses the limitations of the previous stage by focusing on a single, well-known model bacterium with strong relevance to bioelectrochemical systems, namely *Shewanella oneidensis* MR-1. This organism is known for extracellular electron transfer and interactions with various electron acceptors. These characteristics make it suitable for mechanistic interpretation and directly connect to later stages of the work based on the use of microbial fuel cells. The aim of this chapter was to build controlled and optimization-based EB biodegradation frameworks. Instead of testing one variable at a time, Response Surface Methodology (RSM) was used to quantitatively determine how key variables affect the rate and completeness of EB biotransformation (Table 1). For this reason, a full factorial  $3^4$  design was applied with four factors: incubation time (24–72 h), EB concentration (25–100 mg/L), glucose concentration (250–1000 mg/L), and temperature (25–37 °C). These variables are the main controlling factors in biological azo dye conversion. Time controls exposure and reaction completion. Temperature affects microbial growth and enzyme activity. Dye concentration affects toxicity and electron demand. Electron donor availability controls the reducing power needed for azo bond reduction and later metabolic stages.

UV–Vis spectrophotometry was used to monitor sample decolorization, but the results of this method were not treated as proof of biodegradation. Chemical transformation was confirmed using additional analytical tools. HPLC was used to track the disappearance of the parent dye,

providing direct evidence that EB is removed and not only changes its spectral form. GC–MS was used to identify biotransformation products, showing that new compounds appear as EB breaks down. This combined approach strengthens interpretation because it separates color loss resulting from azo bond cleavage from more advanced chemical transformation. It also supports pathway interpretation by linking parent dye reduction with the appearance of identifiable products.

Combining RSM and the obtained process window with chemical analyses provides a documented operating window rather than only an analysis of one factor. This is important because biological dye treatment often depends on interactions among variables. For example, more electron donor can accelerate reduction of azo bonds, but it can also change biomass “behavior,” resulting in a decrease in dye removal efficiency. Higher temperature can accelerate kinetics but may affect stabilization of the process rate. Higher dye loads increase toxicity and electron demand. Optimization based on statistical RSM analysis and the obtained process window, being a defined range of technological parameters allowing stable and repeatable process performance, is much more useful than optimizing a single parameter. Under optimized conditions, almost complete decolorization of EB was achieved, above 99% in 3 days (Figs. 3–4). More importantly, true degradation was confirmed because disappearance of the parent dye and formation of products of further biotransformation of aromatic amines were observed. A significant shortening of treatment time was achieved compared with the experiment based on immobilization of actinomycete biomass. The time to complete decolorization shortened from about 14 days in the carrier-based work to about 3 days in the optimized pure-culture work. Moreover, when process and growth conditions were fully optimized, the pure culture of *S. oneidensis* MR-1 completely mineralized EB.

UV–Vis spectra (400–800 nm) for Evans Blue (Figure 3) in a sample with *S. oneidensis* MR-1 at a concentration of 50 mg/L and a temperature of 30 °C, collected after 2, 4, 6, 8, 11, 14, 24,

28, 48, and 72 h. The characteristic peak around 606 nm gradually disappears, indicating decolorization over time (K. Ayaz et al. 2026a).

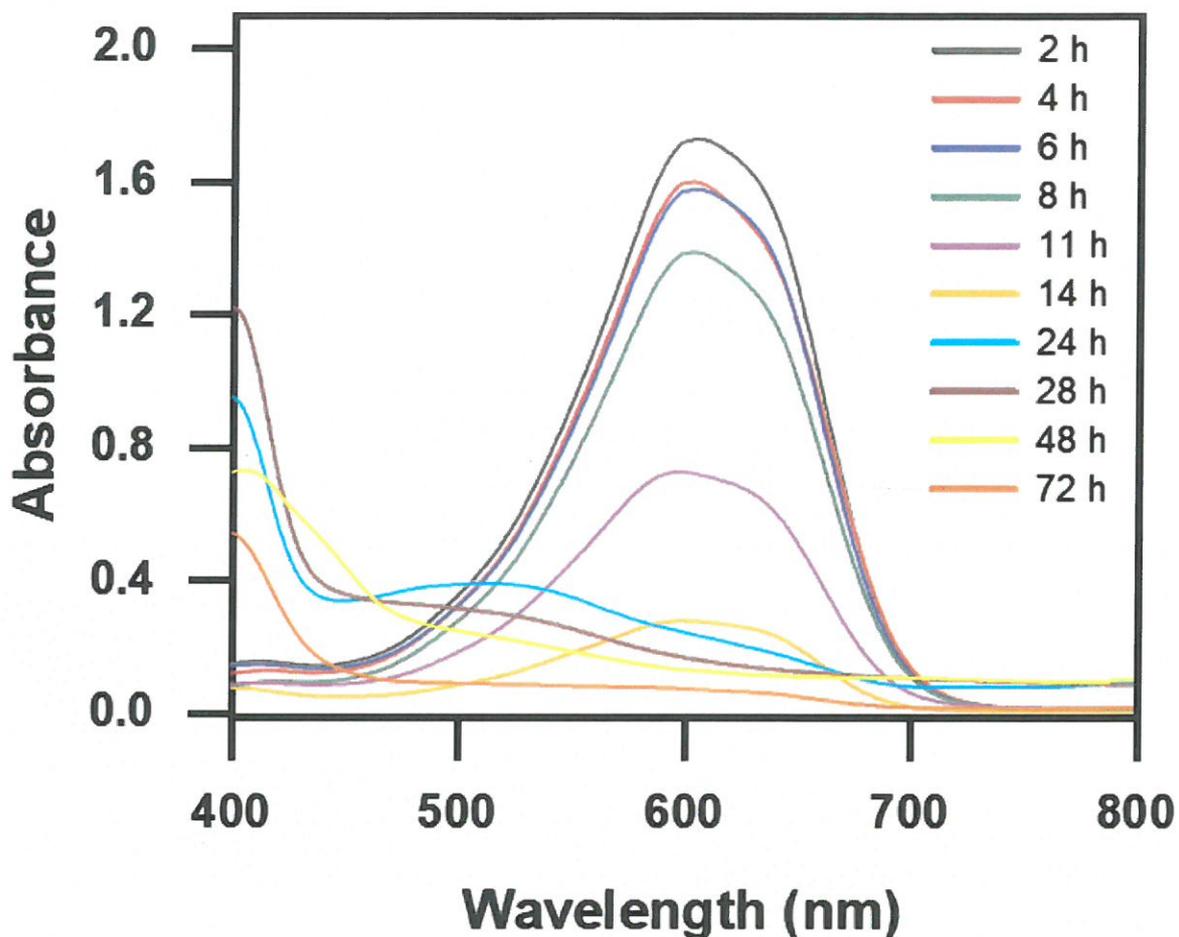


Figure 3. UV-Vis spectra (400–800 nm) of Evans blue during treatment with *S. oneidensis* MR-1 at 50 mg/L and 30 °C, sampled at 2, 4, 6, 8, 11, 14, 24, 28, 48, and 72 h. The characteristic visible band near 606 nm decreases progressively, indicating decolorization over time (K. Ayaz et al. 2026a).

Decolorization of Evans Blue by *Shewanella oneidensis* MR-1 at different glucose concentrations, dye concentrations, incubation times, and temperatures (Figure 4). Panels (a–c) show results for glucose concentration 250 mg/L, (d–f) 500 mg/L, and (g–i) 1000 mg/L. Each panel shows residual dye concentrations (mg/L) after 24, 48, and 72 hours at three initial dye concentrations (25, 50, and 100 mg/L) and temperatures (25, 30, and 37 °C). The dashed

line represents the abiotic control, while the solid line represents degradation by *Shewanella oneidensis* MR-1 (K. Ayaz et al. 2026a).

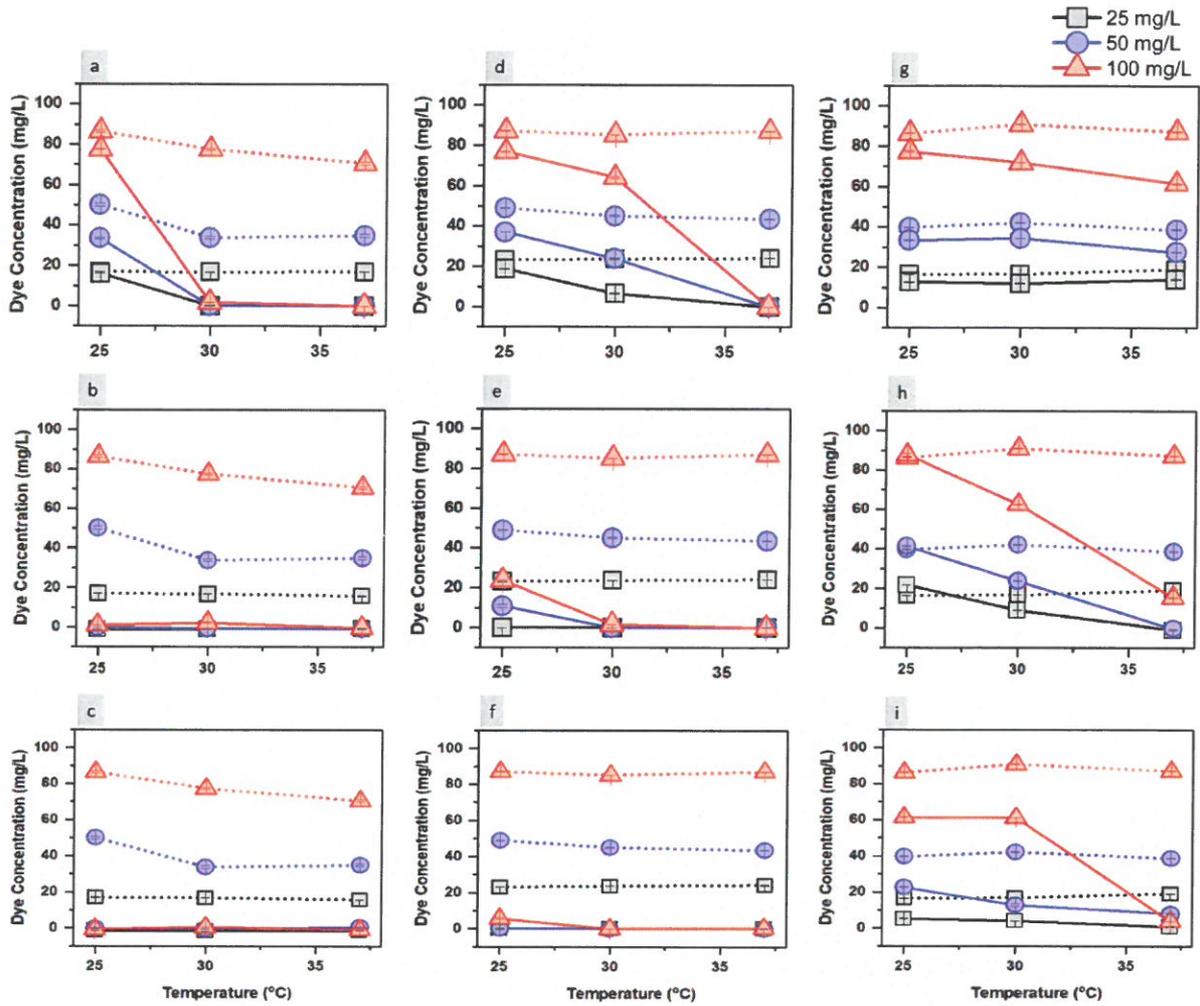


Figure 4. Decolorization of Evans blue by *Shewanella oneidensis* MR-1 at various glucose concentrations, dye concentrations, incubation times, and temperatures. Panels (a–c) represent the results at 250 mg/L glucose, (d–f) 500 mg/L, and (g–i) 1000 mg/L. Each panel shows the residual dye concentrations (mg/L) after 24, 48, and 72 h at three initial dye loads (25, 50, and 100 mg/L) and temperatures (25, 30, and 37 °C). The dotted line represents the abiotic control, whereas the solid line represents *Shewanella oneidensis* MR-1 degradation (K. Ayaz et al 2026a).

Table 1 Analysis of variance (ANOVA) results for the decolorization of Evans blue by *Shewanella oneidensis* MR-1 (K. Ayaz et al. 2026a)

Term	Sum of Squares	DF	F value	P value	Significance
<b>Model</b>	<b>79 371.91</b>	<b>14</b>	<b>17.32</b>	<b>&lt;0.001</b>	<b>Significant</b>
Time (A)	18 339.06	1	56.04	<0.001	Significant
Dye (B)	1 091.33	1	3.33	0.072	Borderline
Carbon source (C)	28 571.05	1	87.30	<0.001	Significant
Temperature (D)	18 700.79	1	57.14	<0.001	Significant
A <sup>2</sup>	2 021.90	1	6.18	0.015	Significant
B <sup>2</sup>	100.49	1	0.31	0.581	Not significant
C <sup>2</sup>	1 015.80	1	3.10	0.083	Borderline
D <sup>2</sup>	596.50	1	1.82	0.182	Not significant
A×B	14.28	1	0.04	0.835	Not significant
A×C	182.48	1	0.56	0.458	Not significant
A×D	4 215.23	1	12.88	<0.001	Significant
B×C	429.94	1	1.31	0.256	Not significant
B×D	216.40	1	0.66	0.419	Not significant
C×D	1 221.96	1	3.73	0.058	Borderline
<b>Residual (Error)</b>	<b>21 599.01</b>	<b>66</b>	—	—	—
<b>Total</b>	<b>100 970.91</b>	<b>80</b>	—	—	—

The quadratic model was statistically significant ( $R = 0.79$ ; adjusted  $R = 0.74$ ), indicating a good fit to the experimental data (Table 1). Time and temperature were the main positive parameters of EB removal, whereas excess glucose inhibited decolorization, and dye concentration had a smaller effect within the tested range. This result supports a key point of the dissertation. A pure culture can provide deep conversion when operating conditions are properly defined and when evidence is supported by chemical analyses rather than only color loss. This chapter therefore creates a strong basis for moving to bioelectrochemical systems because it establishes a model organism, a statistically supported process window, and a verification strategy that links decolorization with actual chemical transformation.

In the third experimental chapter, the dissertation focused on a more engineering-based treatment application. EB removal was studied in an integrated system that combines a double-chamber microbial fuel cell (DCMFC) with an aerobic bioreactor (Figure 5). This chapter addressed the practical reality that industrial dye wastewater often has higher dye loads and more complex composition than simplified laboratory tests. The system design considered biotransformation steps of azo dyes known from the literature. Anaerobic or low-redox

conditions support reductive cleavage of the azo bond, whereas aerobic conditions support oxidation of reduced intermediates. In the DCMFC, the anode chamber provides a low-redox environment that supports azo bond reduction. At the same time, the MFC enables energy recovery by generating electric current. Then aerobic polishing oxidizes reduced intermediates and remaining organic compounds, supporting complete mineralization.

A mixed anaerobic sludge from a wastewater treatment plant was used as inoculum for the DCMFC anode. EB was tested at initial concentrations of 100 and 200 mg/L. This increases the treatment challenge compared with earlier experiments and tests performance under higher dye stress. In the aerobic stage, two actinomycete strains isolated from garden compost were used. This aerobic community was selected to support oxidation of aromatic intermediates formed during anaerobic reduction of azo dyes. This integrated system was intended to combine rapid decolorization with complete mineralization through process staging.

Interpretation of decolorization results was strengthened with electrochemical analyses and illumina sequencing analyses illustrating changes within the microbial community forming the biofilm on the anode. UV-Vis analyses were used to assess decolorization kinetics. Electrochemical Impedance Spectroscopy (EIS) assessed cell and anode resistances and helped identify charge transfer limitations. Cyclic Voltammetry (CV) provided information about electrochemical activity related to biofilm processes. 16S rRNA sequencing identified the two *actinomycetes* strains (SK-2 and SK-3). Together, these tools link treatment performance with ~~electrochemical processes and community changes, providing a detailed picture of processes~~ occurring in the anode biofilm.

The integrated system showed high EB removal performance (Figure 6a, b), at 100 mg/L, decolorization ranged from  $90 \pm 2\%$  to  $98 \pm 1.9\%$  and at 200 mg/L of EB, decolorization ranged from  $79 \pm 2\%$  to  $87 \pm 1\%$ . The process reduced treatment time from  $\sim 3$  days (optimized pure culture) to  $\sim 20$ – $24$  hours when the aerobic stage was included and supported complete degradation treatment compared with decolorization alone. However, this chapter also identified limitations important for scalability: long start-up time ( $\sim 30$  days), extra energy demand and complexity due to aerobic post-treatment, and more difficult mechanistic tracing due to mixed-community interactions. These limitations motivated the final chapter, which asked whether performance could be intensified in a simpler system by addressing the bioelectrochemical bottleneck at the anode.

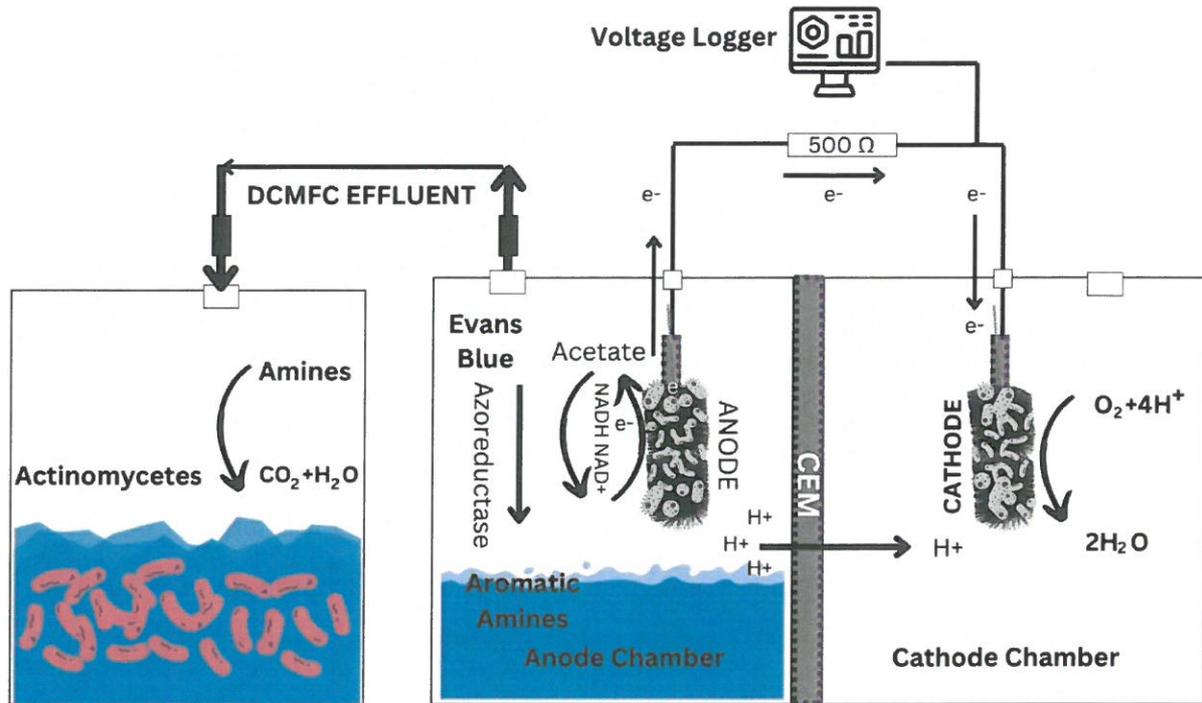


Figure 5. Schematic diagram of double – chamber microbial fuel cell (K.Ayaz et al. 2024)

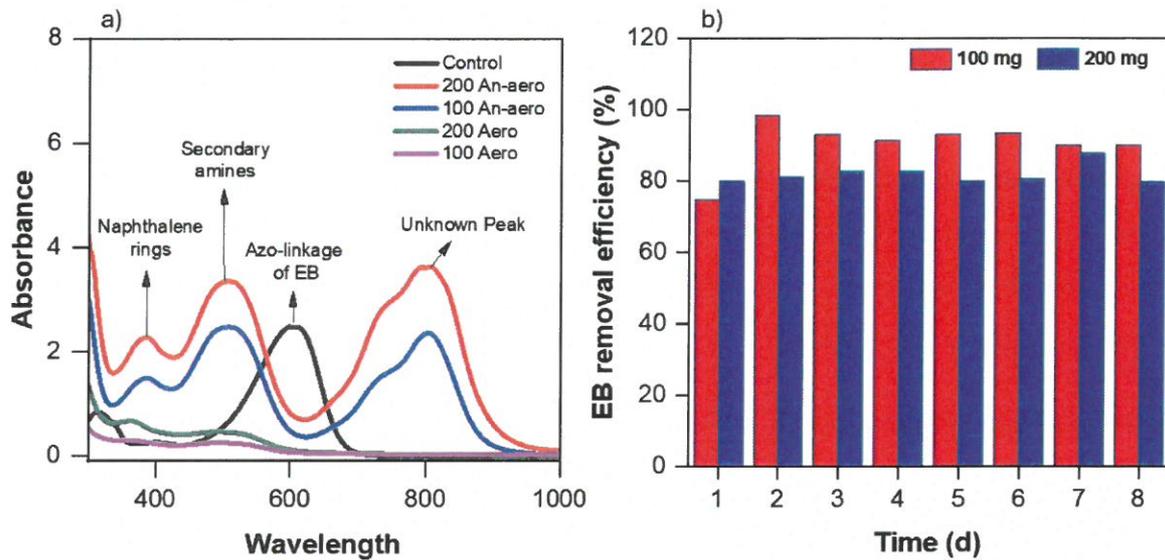


Figure 6. a) UV-visible absorption spectra of 100 mg/L and 200 mg/L of DCMFC anaerobic effluent, pure dye solution (control), and aerobically treated effluent. (b) Decolorization of EB in DCMFC with time (K.Ayaz et al. 2024)

The final experimental chapter focused on solving the above limitations through engineering of the microorganism–electrode interface. In microbial fuel cells, the anode surface controls early adhesion, biofilm growth, and extracellular electron transfer. Poor adhesion delays start-up. High interfacial charge-transfer resistance limits reaction rate and electron recovery. In addition, pure cultures may be more sensitive to dye stress than mixed communities. Therefore, improving the anode surface was treated as a direct engineering tool to shorten start-up,

improve performance, and increase tolerance to higher dye loads while keeping the system simple and easier to interpret.

In this work, a carbon cloth anode was sequentially modified with a polyaniline (PANI) layer, a glucose layer, and a protective gelatin layer (Figure 7). Each layer served a specific role. PANI improved conductivity and supported better charge transport. The glucose layer acted as a local carbon layer that supported early colonization and biofilm formation. The gelatin layer protected glucose and slowed down its exposure also protected from instant dissolution in the surrounding solution to extend its benefits in the early phase. The coating was verified using CV during PANI electropolymerization and by surface analyses such as FTIR, SEM, and XPS. Performance was measured using CV, EIS, and CSC, together with evaluation of biofilm coverage and viability.

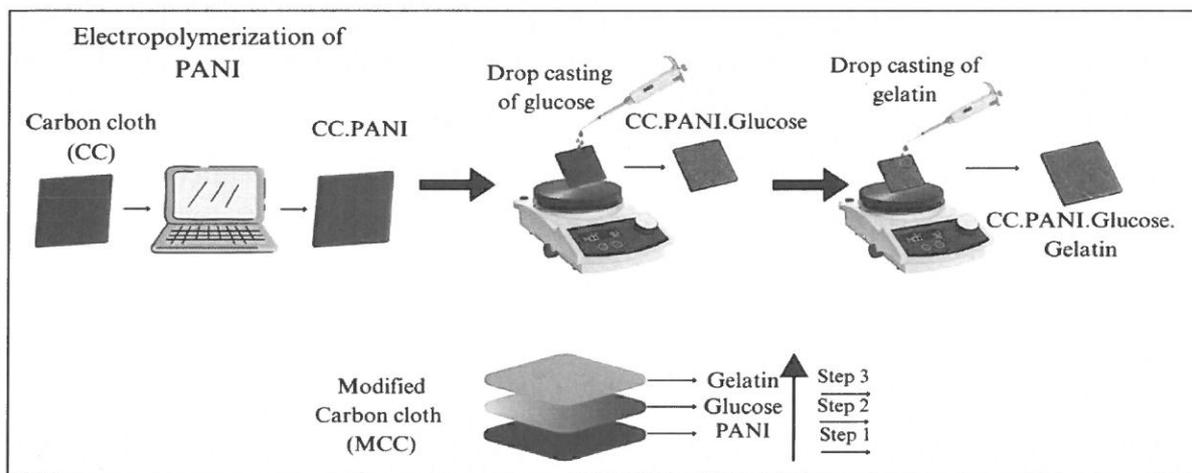


Figure 7. Schematic representation of the electrode modification (K. Ayaz et al. 2026b).

The results showed clear differences between the system with glucose available in the bulk solution and glucose placed on the electrode surface. When glucose was present in solution (CC.G.B), bacteria tended to grow in the liquid phase, which limited stable adhesion. In contrast, the modified anode (MCC.B) showed the highest biofilm formation ( $90.2 \pm 0.2\%$ ) and the highest CSC ( $174.9 \pm 10.2 \text{ mC/cm}^2$ ), which was much higher than for unmodified carbon cloth with biofilm (CC.B,  $3.3 \pm 0.3 \text{ mC/cm}^2$ ). This supports the conclusion that surface modification and conductive coatings improve biofilm growth and reduce interfacial limitations, which improves electron transfer and shortens early-stage development (Figure 8).

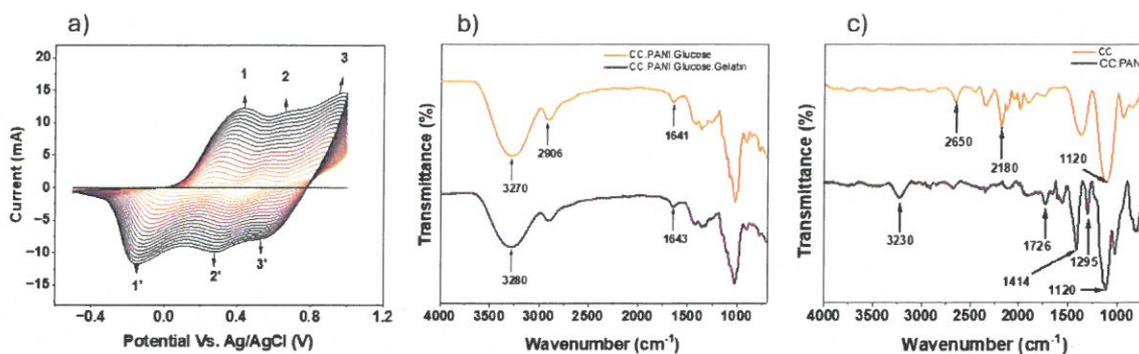


Figure 8. (a) Cyclic voltammogram presenting the electrochemical polymerization of aniline on the surface of carbon cloth. (b-c) FTIR spectra of experimental samples, including (c) carbon cloth (CC), CC electropolymerized with PANI (CC.PANI), and (b) CC-PANI coated with glucose (CC.PANI.Glucose), and a protection layer of gelatin (CC.PANI.Glucose.Gelatin referred to as MCC) (K. Ayaz et al. 2026b).

The modified anode was then tested in a single-chamber MFC (SCMFC), which is simpler than a double-chamber configuration. In the SCMFC, the modified interface achieved about 96–97% decolorization at 150 mg/L in 13 hours, about 90–91% at 300 mg/L in 13 hours, and 73% at 450 mg/L in 13 hours (Figure 9). This demonstrated faster treatment than the DCMFC-based system and a higher dye-load tolerance, reaching 450 mg/L whereas earlier inhibition and probable toxicity were observed above 200 mg/L. GC–MS detected low-molecular-weight acids such as 3-methylbutanoic acid and octanoic acid as extractable end-pool compounds in the anode matrix, supporting that EB was transformed to smaller detectable products. At the same time, GC–MS data indicated degradation to less toxic products, low-molecular-weight acids such as 3-methylbutanoic acid and octanoic acid, and this was achieved without adding an aerobic polishing step. This supports the dissertation goal of moving toward a simpler, faster, and more controllable bioelectrochemical system for deep EB transformation.

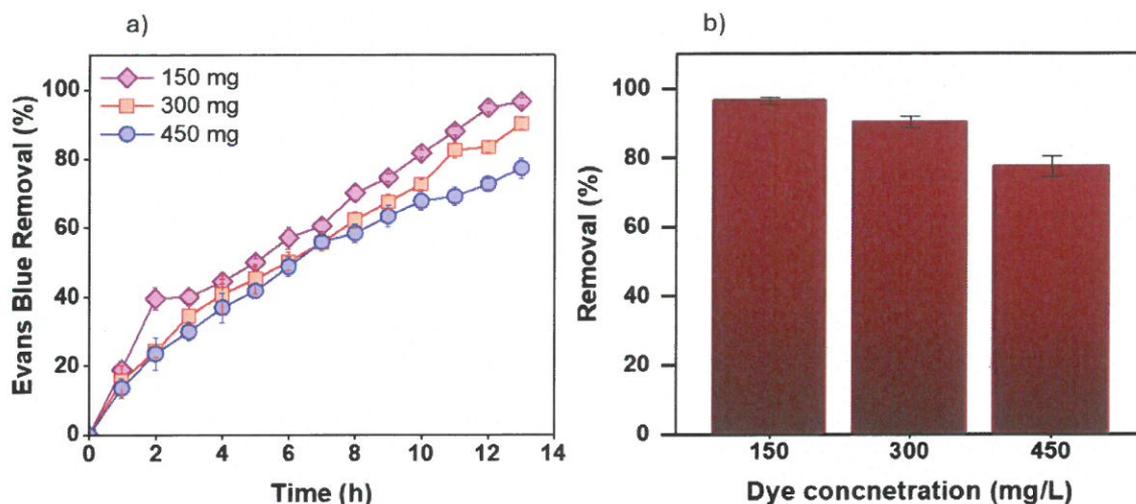


Figure 9. (a) Percent dye removal over time in SCMFC for initial Evans blue dye concentrations of 150, 300, and 450 mg/L (b) Maximum dye removal for all concentrations

In summary (Table 2), the dissertation presents a clear and coherent treatment pathway for azo dyes using the example of the persistent sulfonated azo dye EB. It begins with microbial screening and carrier testing for decolorization of triphenylmethane and azo dyes, which builds a strain resource and highlights the importance of separating adsorption from biodegradation and the issue of long incubation times. It then uses a pure culture and statistical optimization to determine a robust process window and confirms transformation using HPLC and GC-MS. The next stage is DCMFC with a mixed community combined with aerobic post-treatment, which enables rapid treatment and complete mineralization but reveals start-up and complexity constraints. Finally, the work solves the key bioelectrochemical bottleneck through engineering of the anode interface, improving adhesion, electron transfer, start-up performance, and tolerance to higher dye loads in a simpler SCMFC setup. The overall contribution includes not only demonstrating high removal efficiency but also linking biological mechanisms with operational tools, enabling clearer interpretation and more practical deployment of biological and bioelectrochemical wastewater treatment systems.

Table 2 stepwise progress of EB treatment across the dissertation stages, summarizing the tested system/configuration, EB loading range (mg/L), treatment time, decolorization/removal performance, and the key conclusion (from carrier-assisted actinomycete screening to RSM-optimized pure culture, redox-staged DCMFC with aerobic polishing, and SCMFC intensification via engineered anode biointerface).

Experimental Stage	System / Microorganism	Dye Concentration mg/L	Max Decolorization (%)	Time (d/h)	Key conclusion

Stage 1: Screening	Isolated Actinomycetes with straw carrier	45	80 (1K1) 73 (EGK2)	14 d	Removal percentages based on carrier.
Stage 2: Shewanella Optimization	Shewanella oneidensis MR-1 (Single strain)	25–100	>99.0	24– 72 h	GC–MS/HPLC: Formation of aromatic and short-chain acids with complete mineralization
Stage 3: Integrated System	Mixed Actinomycetes in DCMFC–Aerobic	100 200	98.0 87.0	20-24 h	16S rRNA: Pseudomonadota dominance; complete mineralization
Stage 4: Electrode Engineering	SCMFC with Modified Carbon Cloth (MCC.B)	150 300 450	96.56 90.22 77.28	13 h	Rapid EB removal, GC–MS: 3-methylbutanoic and octanoic acid with incomplete mineralization

## 1. References to the author's main publications

This dissertation relates to thematically linked publications:

- Ayaz, K., Zabłocka-Godlewska, E., Shakibania, S., Patel, T., Karoń, K., & Krukiewicz, K. (2026b). Enhancing Microbial Adhesion and Electron Transfer of *Shewanella oneidensis* MR-1 with Polyaniline-Glucose-Gelatin Coating for Bioelectrochemical Applications. *Journal of Environmental Chemical Engineering*. <https://doi.org/10.1016/j.jece.2026.121546>
- Ayaz, K., Zabłocka-Godlewska, E., Smółka, S., Kudlek, E., Hussain, I., & Ilyas, M. (2026a). Sustainable biodegradation of Evans blue (Direct Blue 53) by *Shewanella oneidensis* MR-1: Response surface optimization and product analysis. *Journal of Water Process Engineering*. <https://doi.org/10.1016/j.jwpe.2025.109399>
- Ayaz, K., Zabłocka-Godlewska, E., & Li, C. (2024). Enhancing Azo Dye Mineralization and Bioelectricity Generation through Biocathode-Microbial Fuel Cell Integration with Aerobic Bioreactor. *Energies*, 17(19), 4896. <https://doi.org/10.3390/en17194896>
- Ayaz, K., & Zabłocka-Godlewska, E. (2022). Screening of Actinomycetes decolorizing the synthetic dyes from rotten poplar wood and garden compost. In *Contemporary Problems of Power Engineering and Environmental Protection* (pp. 119–131).