Abstract 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***

Abstract

Synthetic dyes are widely used, especially in textiles, because they give strong color, many shades, and good fastness. A large portion of the applied dye does not bind to fibers and washes out, creating colored wastewater. This is even worse for highly water-soluble, anionic dyes that are built to stay stable in dye baths. Their stability, charge, and structure make them hard to remove with standard physical, chemical, or biological treatment. Azo dyes are the largest group and are often difficult to treat. Upon breaking up of Azo bond removes color quickly but can form aromatic by-products that may persist or need aerobic oxidation to lower toxicity. So, treatment must go beyond decolorization to prove real chemical change, manage by-products, and work across different microbes and reactor designs.

This dissertation examines biological decolorization of several synthetic dyes, but most of the work uses Evans Blue (EB; Direct Blue 53) as the main model compound. EB is a highly sulfonated diazo dye that dissolves easily in water, produces an intense colour, and represents the type of anionic azo dye that is difficult to treat in practice. The research follows a staged approach. It starts with tightly controlled biological experiments and then moves toward engineered bioelectrochemical systems that can operate faster and handle higher loads.

Across all stages, treatment is evaluated using mechanistic and chemical evidence, not only UV–Vis estimated color loss. The aim is to build and validate biological and bioelectrochemical routes that accelerate dyes, mainly EB removal with both pure and mixed cultures, while also demonstrating that the dye is chemically transformed rather than temporarily masked by adsorption or partial reduction. The dissertation also makes clear where each approach reaches its limits and identifies the design and operating changes that most effectively improve performance and control

The first experimental chapter focuses on the isolation of selected bacterial species (*Actinomycetes*) involved in decolorization processes. *Actinomycetes* were screened on solid media

for dye decolorization, with EB as the target azo dye and Brilliant Green (BG) and Crystal Violet (CrV) as triphenylmethane comparators. In this part of studies, biomass immobilization at low-cost solid carriers were evaluated to stabilize biomass, increase retention, and support later translation to reactor systems. Low-cost solid carriers were tested to optimize the isolated microbe's growth and degradation efficiency. The carriers used were conifer shavings, straw, and leafy chips. Only straw supported visible microbial growth in initial testing, so we selected the straw for the main experiment as well. After 14 days, straw alone and biomass showed less decolorization or degradation, while combining straw with biomass showed better results. Strain 1K1 removed 66.6% of BG and 80% of EB. Strain EGK2 removed 97.8% of BG and 73% of EB. A key goal was to separate the main mechanism of color loss. In carrier systems, color can be lost because of biosorption, microbial degradation or because dye adsorbs when carriers are introduced. The screening results helped us pick strains for aerobic post-treatment in later experimental chapters. Despite better dye removal there were few limitations that need to be addressed. Long incubation times and mixed physical and biological effects were the main issues. These findings support moving to another model organisms and tighter, controlled optimization in the next experimental chapters.

The second experimental chapter examines EB biodegradation by the electroactive model bacterium *Shewanella oneidensis* MR-1. The design uses Response Surface Methodology (RSM) to optimize key variables: time, temperature, initial dye concentration, and electron-donor concentration. UV–Vis kinetics are used to tracked color loss. While chemical transformation was checked by chromatographic technique HPLC to tracked parent dye loss and GC–MS for the identification of transformed products. This combined approach defines a defensible operating window rather than a single operating point. It clarifies how dye load, temperature, and electron-donor supply control both the rate and the completeness of Evans Blue (EB) bioconversion. Under optimized conditions, nearly complete decolorization (>99%) was achieved. Loss of the chromophore, together with the appearance of aromatic and short-chain acid products, confirmed true dye degradation rather than biosorption. These findings show that *Shewanella oneidensis* MR-1 is an effective single-strain biocatalyst for EB removal and that response surface methodology (RSM) can identify a practical operating window for future applications. This work also reduced the time to complete decolorization from 14 days (experimental section 1) to 3 days. In addition,

the pure culture of *S. oneidensis* MR-1 completely mineralized EB when process and growth conditions were fully optimized.

The third experimental chapter investigates efficient decolorization, degradation and complete mineralization of the diazo dye EB using an integrated system that couples a double-chamber microbial fuel cell (DCMFC) with an aerobic bioreactor. This work targets solutions to problem of high concentrations of toxic dyes, with two goals: achieving high decolorization efficiency and characterizing the microbial communities that drive decolorization and degradation. Mixed anaerobic activated sludge from a wastewater treatment plant was used to evaluate EB removal in the DCMFC anode at initial dye concentrations of 100 and 200 mg/L. In the MFC, the anode creates a low-redox environment that promotes reductive cleavage of azo bonds while enabling energy recovery as electrical current. The aerobic post-treatment step employed two mixed actinomycete strains isolated from garden compost. These *Actinomycetes* consortia oxidized the reduced intermediates and residual organics produced at the anode, supporting full mineralization of the dye.

Decolorization efficiency and microbial community composition were evaluated using 16S rRNA sequencing, and electrochemical impedance spectroscopy (EIS) was used to assess anode and DCMFC resistance. The results demonstrated decolorization efficiencies ranging from 90 ± 2% to 98 ± 1.9% for 100 mg/L and from 79 ± 2% to 87% ± 1% for 200 mg/L after 20-24 h of process. The microbial community analysis revealed a significant presence of *Pseudomonadota* (45.5% in dye-acclimated cultures and 32% in inoculum cultures), with key genera including Actinomarinicola (13.75%), Thermochromatium (4.82%), and *Geobacter* (4.52%). This study highlights the potential of the integrated DCMFC–aerobic system, utilizing mixed *Actinomycetes* strains for the very first time in aerobic step, what may be beneficially applicated in the future for the effective treatment of industrial dye effluents, offering both environmental and bioenergy benefits.

This chapter presents the solutions that cut decolorization time from 3 days to 20–24 hours and achieved complete mineralization by adding a secondary aerobic step. It also treated a higher starting Evans Blue load of 200 mg/L, double that used in experimental chapters 1 and 2. Three limits remain: a long start-up period (adaptation) of about 30 days before we get start the actual experiment of degradation of EB with initial concentration of 100 and 200mg/L, extra energy

demand for the aerobic step, and difficult mechanism tracing with a mixed community. These issues point to the need to optimize the reactor and MFC biointerface and attempt to utilize environmentally friendly single chamber microbial fuel cell (SCMFC) so that pure culture can match or exceed this performance while giving clearer mechanisms and easier process control.

Based on the limitation of previous chapter, the final experimental chapter investigates the effect of carbon cloth modifications (anode electrode) using polyaniline (PANI), glucose, and gelatin on biofilm formation, charge transfer, and microbial viability using pure bacterial culture of *Shewanella oneidensis* MR-1. The Pure culture is more prone to toxic effect of dyes and underperformed in high initial dyes concentration. Cyclic voltammetry results and Biofilm viability studies reveal that carbon cloth in bulk glucose solution (CC.G.B) supports predominantly planktonic growth, limiting bacterial adhesion, while modified carbon cloth (MCC.B) achieves the highest biofilm formation (90.2 ± 0.2 %) and charge storage capacity (CSC, 174.9 ± 10.2 mC/cm^2), corresponding to an approximately 50-fold increase in CSC compared with a bare carbon cloth (CC.B) system (3.3 ± 0.3 mC/cm^2), highlighting the synergistic impact of surface modifications and microbial activity. These findings demonstrate that MCC.B facilitates extracellular electron transfer by optimizing bacterial adhesion and nutrient availability, making it a promising candidate for shortening start-up to 3 days (30 days in experimental section 3) and improving early-stage bioelectrode performance.

In the SCMFC, the modified carbon cloth anode interface achieved about 96–97% decolorization at 150 mg/L in 13 hours ($t50 \approx 5$ h; $t80 \approx 9$–10 h), 90–91% at 300 mg/L, and 73% at 450 mg/L in 13 hours. Thus, lowering the decolorization time to 13 hours from 24 hours (experimental section 3). The system also operated stably at an initial concentration of 450 mg/L, whereas earlier experiments showed performance limits because of the dye toxicity above 200 mg/L. While GC-MS analysis showed that EB are degraded to less toxic compounds mostly low molecular weight acids 3-methylbutanoic and octanoic acid even without post-treatment aerobic step. These outcomes show a clear, controllable sequence, modified surface chemistry of anode electrode improved bacterial attachment of pure bacterial culture by bearing the toxic effect of high initial dye concentration through well-developed biofilm on modified electrode. This also strengthened electron transfer and increased process rate and capacity. The approach remains simple, uses standard carbon cloth, and is scalable for faster treatment.