Environmental Pollution 335 (2023) 122253

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Molecular characterization of azoreductase and its potential for the decolorization of Remazol Red R and Acid Blue 29☆

Ghulam Mustafa [a,b], Muhammad Tariq Zahid [b], Mayur Bharat Kurade [a], Swapnil Mahadeo Patil [a], Farah Rauf Shakoori [c], Zeeshan Shafiq [b], Sidra Ihsan [b], Yongtae Ahn [a], Azmat Ali Khan [d], Amel Gacem [e], Byong-hun Jeon [a,*]

[a] Department of Earth Resources and Environmental Engineering, Hanyang University, Seoul, 04763, South Korea
[b] Department of Zoology, Government College University, Lahore, 54000, Lahore, Pakistan
[c] Institute of Zoology, University of the Punjab Lahore, Pakistan
[d] Pharmaceutical Biotechnology Laboratory, Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, Riyadh, 11451, Saudi Arabia
[e] Department of Physics, Faculty of Sciences, University 20 Août 1955, Skikda, 21000, Algeria

ARTICLE INFO

Keywords:
Decolorization
Remazol red
Azoreductase
Biodegradation
Bioremediation

ABSTRACT

Azoreductase is a reductive enzyme that efficiently biotransformed textile azo dyes. This study demonstrated the heterologous overexpression of the azoreductase gene in *Escherichia coli* for the effective degradation of Remazol Red-R and Acid-Blue 29 dyes. The *AzK* gene of *Klebsiella pneumoniae* encoding a ≈22 kDa azoreductase enzyme was cloned into the pET21^{+}C expression vector. The inoculum size of 1.5%, IPTG concentration of 0.5 mM, and incubation time of 6 h were optimized by response surface methodology a statistical tool. The crude extract showed 76% and 74%, while the purified enzyme achieved 94% and 93% decolorization of RRR and AB-29, respectively in 0.3 h. The reaction kinetics showed that RRR had a K_m and V_{max} value of 0.058 mM and 1416 U mg^{-1}, respectively at an NADH concentration of 10 mM. HPLC and GC-MS analyses showed that RRR was effectively bio-transformed by azoreductase to 2-[3-(hydroxy-amino) benzene-1-sulfonyl and AB-29 to aniline and 3-nitrosoaniline. This study explored the potential of recombinant azoreductase isolated from *K. pneumoniae* in the degradation of toxic textile azo dyes into less toxic metabolites.

1. Introduction

Water Pollution is an emerging concern and an immediate threat to aquatic life (Schweitzer and Noblet, 2018). Globalization has caused a rapid shift in the textile and garment industries in both developed and developing countries for more than twenty-five years, contributing nearly 60% of clothing export and apparel currently being produced in the latter. This sector is the biggest user of dyes and pigments worldwide, with up to 50% of total production (Raman and Kanmani, 2016). The treatment of massive amounts of textile wastewater released into the environment because of the rapid expansion of this industry has been a significant concern (Chandanshive et al., 2016; Samuchiwal et al., 2023). Textile dyes are xenobiotic, mutagenic, and carcinogenic (Morrison et al., 2012). The Azo dyes are contributing >50% of annual dyes production worldwide (Brüschweiler and Merlot, 2017).

There is a myriad of chemical and physical dye removal techniques, including membrane extraction, coagulation/flocculation, precipitation, adsorption, oxidation, and advanced oxidation processes (Waghmode et al., 2019). These methods are effective in removing color from wastewater; however, they face several challenges, such as the production of a large quantity of slurry, which results in secondary pollution issues; high operation costs, difficult processes, the production of a sizable amount of sludge, reduced economic viability partial removal of azo dyes and their metabolites rather than degradation (Saratale et al., 2013; Kurade et al., 2015; Ihsanullah et al., 2020). Importantly, chemical-intensive techniques produce undesired metabolites that are more toxic than parent compounds. The concentrated contaminants require further post-treatment or management which increases the cost of wastewater treatment (Parmar and Shukla, 2018).

Biological approaches are seen as precise, less energy-demanding, productive, and eco-friendly due to the efficient degradation of organic contaminants into stable and harmless end products (Kurade

☆ This paper has been recommended for acceptance by Sarah Harmon.

* Corresponding author.
E-mail address: bhjeon@hanyang.ac.kr (B.-h. Jeon).

https://doi.org/10.1016/j.envpol.2023.122253
Received 2 March 2023; Received in revised form 12 July 2023; Accepted 22 July 2023
Available online 25 July 2023