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EXPLORING THE BIODEGRADATION POTENTIAL OF CRUDE EXTRACELLULAR ENZYME MIXTURE FROM *ANOXYBACILLUS* SP. FOR AZO DYE REMOVAL: A SUSTAINABLE APPROACH FOR INDUSTRIAL WASTEWATER TREATMENT

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Abstract

Urbanization, globalization, and industrialization have resulted in the accumulation of environmental contaminants such as organic chemicals, metals, and dyes. The textile industry is a significant contributor to environmental contamination due to the emission of wastewater containing harmful reactive and azo dyes, which are known mutagens, carcinogens, and teratogens. Several microorganisms, including bacteria, algae, fungi, and yeasts, can effectively degrade industrial dyes; however, their efficacy significantly depends on their adaptability and activity. This study investigated the potential of crude extracellular enzyme preparation from thermophilic indigenously isolated *Anoxybacillus* sp. in the biodegradation of azo dyes at high temperatures. This could be an innovative method for treating wastewater contaminated with azo dyes, including RB5, Methyl Orange, and Congo Red. The crude enzyme was isolated from *Anoxybacillus* sp. and its effect was investigated *in vitro* at a pH and temperature range of 4-8 and 20-60°C, respectively. The residual concentrations of selected dyes were measured using a UV-Vis spectrophotometer. The results demonstrated that the *Anoxybacillus* sp. crude enzyme effectively decolorized the azo dyes, indicating its potential for industrial applications. Additionally, the crude enzyme retained its decolorization capacity even at high temperatures and low pH. The highest decolorization occurred at 60°C for all three dyes and a pH of 4.This study provides a basis for the large-scale production and application of extracellular, thermostable, crude enzyme mixtures using a low-cost substrate. The dye-decolorization efficiency of this bacterial isolate makes it an environment-friendly, low-cost, and effective option for industrial use.

Keywords: Azo dyes, Crude extracellular enzyme, Decolorization, *Anoxybacillus* sp., Thermophile

Introduction

The release of industrial effluents containing azo dyes into the environment has emerged as a significant concern, owing to their harmful impact on human health and the ecosystem (Patel et al. 2022). Among the contaminants found in textile industry effluents, azo dyes are of particular concern (Patel et al. 2022). Azo dyes are synthetic organic compounds that comprise one or more azo (-N=N-) groups (Coughlin et al. 1999; Saratale et al. 2011). They are simple to use, have brilliant colors, and are commonly employed in the textile industry. However, they are also highly toxic, mutagenic, teratogenic, and carcinogenic (Al-Tohamy et al. 2020; Siddiqui et al. 2023), posing a considerable hazard to human health, society, and the environment. They exhibit poor biodegradability, leading to their persistence in the environment (Oladoye et al. 2022).Azo dyes are not only resistant to biodegradation but can also produce harmful byproducts such as aromatic amines and mutagenic compounds like 1, 4-phenylenediamine, and o-tolidine upon breakdown (Siddiqui et al. 2023; Sudha et al. 2014). These toxic byproducts affect the environment and human health. Hence, developing effective and sustainable techniques for eliminating azo dyes from textile effluents is crucial.

The conventional methods of treating wastewater are often expensive and have limited effectiveness (Pandey et al. 2007). Therefore, there is a need for environmentally friendly and economically viable ways to handle industrial effluent. Microbial enzyme-assisted biodegradation of azo dyes has emerged as a promising approach for wastewater treatment (Bhandari et al. 2021). Compared to physicochemical methods, which often generate large amounts of sludge and require significant amounts of energy and resources (De Campos Ventura-Camargo and Marin-Morales 2013; Eltaboni et al. 2022), microbial or enzymatic treatment procedures are more effective and environmentally friendly. They offer advantages, such as reduced chemical use and sludge production, lower water and energy consumption, cost-effectiveness, and non-toxic or fully mineralized end products (Selvaraj et al. 2021). Additionally, enzymes are highly specific and can target particular pollutants, making them an efficient and sustainable solution for wastewater treatment (Şaşmaz et al. 2010).

The selection of bacteria for decolorization depends on their ability to adapt to the specific dye and their activity in breaking it down. Different species of bacteria have been studied to determine their effectiveness in decolorizing and mineralizing different types of azo dyes (Shah 2014; Singh and Singh 2017).The use of bacterial strains that can completely decolorize and degrade azo dyes, such as *Xanthomonas* sp. NR25-2, *Bacillus* sp. OY1-2, *Anoxybacillus* sp., and *Pseudomonas* sp. PR41-1, through microbial or enzymatic treatment procedures, has several advantages (Bhandari et al. 2021; Ferraz et al. 2011; Pal et al. 2022). However, although enzymes from several of these organisms have been studied, particularly laccase enzymes (Shah et al. 2023; Wang et al. 2020) and their decolorization properties, studies on the crude enzyme extract of *Anoxybacillus* sp. are scarce.

Anoxybacillus sp. is a thermophilic bacterium that produces various enzymes with potential industrial applications operating at high-temperature and pH environments, thus making it a suitable species for industrial wastewater treatment (Deive et al. 2010). The crude extracellular enzymes produced by *Anoxybacillus* sp. have been shown to have high activity against azo dyes, making it a potential tool for their removal from wastewater (Yanmış et al. 2016). However, only a few studies exist on its decolorization capacity. In this study, we explore the potential of a crude extracellular enzyme derived from *Anoxybacillus* sp. for the biodegradation of three well-known azo dyes (RB 5, Congo Red, and Methyl Orange) in industrial wastewater.

Materials and Methods

Materials required

This study investigates the potential of a crude extracellular enzyme to biodegrade azo dyes in wastewater. The enzyme was derived from *Anoxybacillus* sp. indigenously isolated from Manikaran, Kullu district of Himachal Pradesh, India. Three dyes were used for decolorization experiments: Congo red and Methyl Orange, purchased from Himedia (Mumbai, India), and RB5, purchased from MP Biomedicals (IIIkirch, France). The concentration of each dye was selected after initial screening, and a 50-ppm dye solution,each in a 100 ml conical flask,was used for the experiments. To avoid nutrient loss, all the dye solutions were made in filter-sterilized (0.2 µm Merck) dye decolorization broth. Additionally, working solutions were prepared using sterilized decolorization media to account for any potential nutrient loss.

Collection of water sample

Water samples were collected from geothermal hot springs of Manikaran (32.0268° N, 77.3511° E), Himachal Pradesh's Kullu district (India). Manikaran hot springs are located at an elevation of 1,760m above sea level in the Beas and Parvati Valley geothermal system. The temperature of the hot springs ranges from 89 to 99°C. The water samples were collected in thermos flasks beneath the surface, away from the margin and transported to the laboratory within 24hr and screened immediately for isolation (Panda et al., 2013. Isolated samples were stored at 4°C for up to 5-7 days after screening for further screening.

Isolation of Thermophilic bacteria

Water samples collected from the hot springs were serially diluted using sterile saline buffer (Aneja, K.R., 2007). An aliquot of each suspension was spread on nutrient agar (NA) plates containing 0.5% NaCl, 0.4% beef extract, 0.5% peptone, 0.2% yeast extract, and 2% (w/v) agar using the streak plate method; and the pH of the medium was adjusted to 7.0 before autoclaving and incubated at 62°C overnight. After multiple transfers using the same solid medium, morphologically distinct colonies were selected, and pure and active colonies were produced. The obtained pure cultures were maintained in glycerol stock at -80°C for future use. Sigma-Aldrich (USA), Merck (USA), HiMedia Labs (India), and other suppliers provided all of the chemicals, media, media components, and other reagents (Panda et al., 2013).

Organism used for crude extracellular enzyme production

Anoxybacillus sp. was selected as the organism for crude extracellular enzyme production in this study. This bacterium is known for its ability to produce crude extracellular enzymes commonly used in industrial processes such as bioremediation and pulp and paper production (Acer et al. 2015; Goh et al. 2014); however, only a few studies exist on its decolorization capacity. To ensure the purity and stability of the bacterial strain, the species was regularly sub-cultured on nutrient agar (NA) plates at 62°C every 2-3 weeks. NA is an agar medium containing dextrose and peptone as carbon and nitrogen sources. The plates were stored at 4°C to maintain the viability of the bacterial culture over an extended period (Zeng et al. 2011). These procedures are essential to ensure consistency in the quantity and quality of crude extracellular enzymes produced by *Anoxybacillus* sp.

Protein precipitation

Solid Ammonium Sulphate was slowly added to reach the lower percent saturation with careful stirring to prevent overshooting the target concentration. Then it was allowed to precipitate for approximately 30 minutes to balance between reaching equilibrium and maintaining procedural efficiency. All the operations was performed in an ice bucket. Then, the solution was centrifuged at approximately 10,000 × g for about 10 minutes in a pre-cooled rotor to pellet the insoluble material. Supernatant was carefully poured off and volume was measured. to determine the grams of Ammonium Sulphate needed to transition from the lower to the final higher percent saturation we referred to Wingfield P (May 2001). Ammonium Sulphate was slowly added with rapid mixing to avoid high local concentrations and allowed the solution to sit for 30 minutes to facilitate precipitation. Again, the solution was centrifuged at approximately 10,000 × g for about 10 minutes in a pre-cooled rotor. Allow the pellet to drain for about 1 minute to remove excess supernatant. The obtained protein was dissolved in an appropriate lysis buffer (pH 7.4) and after desalting it was stored in -20°C for further experiment.

Dye decolorization assay

In this study, 75ug of the crude extracellular enzyme extract was utilized to investigate the decolorization process of RB5, Congo Red, and Methyl Orange dyes. The spectrophotometer (UV-1800, Shimadzu) was set to the respective maximum wavelengths of the dyes (λ_{max}: 597nm, 497nm, and 464nm) to measure the rate of dye decolorization (Moilanen et al.2010; Yeşilada et al. 2014). Decolorization was examined by mixing the crude extracellular extract with 50ppm of the dye solutions in citrate phosphate buffer at various pH levels (4-9) and temperatures (20-60°C) in 100 ml conical flasks (Ratanapongleka and Phetsom 2014).After every 12-hour interval, aliquots were analyzed using a UV-Vis spectrophotometer to determine the residual dye concentrations at their respective wavelengths. To further investigate the effect of pH and temperature, 75ugof free crude extracellular enzyme extract was mixed with the dye solutions and citrate phosphate buffer over a pH range of 4-8 and temperature of 20-60°C. All experiments were performed in triplicate, and the mean value was reported. Absorbance readings were taken at 12-

hour intervals until equilibrium was achieved. The percentage of decolorization was calculated by comparing the absorbance of the medium after dye decolorization to the initial absorbance (A_0) of control samples that were not treated with the crude enzyme, using the formula: Decolorization = (A_0 - A_t) / A_0, where A represents the absorbance of the medium after the dye has been decolorized.

% Decolorization = $A_0 – A_t / A_0 \times 100$

Where,

A_0 = initial absorbance

A_t = final absorbance.

UV–Visible (UV-Vis)spectrophotometric analysis

The UV-Vis spectrophotometer was used to monitor the decolorization of RB5, Methyl Orange, and Congo Red dyes. A 100 mL conical flask was used to combine 75 µg of crude extracellular extract at varying pH levels with a 50-ppm dye solution, with constant agitation. After every 12-hour interval, aliquots were taken from the reaction mixture to measure the remaining concentrations of RB5, Congo Red, and Methyl Orange at their respective λmax values (λ_{max}: 597nm, 497nm, and 464nm). The absorption spectrum of the reaction solution was recorded to determine the rate of dye decolorization over time (Yeşilada et al. 2014).

Results

The reaction mixtures were assessed for upto 72 hours at 12-hour intervals. The standard assay conditions, which were previously described above, were maintained throughout the experiment. Decolorization occurred at 72 hours in all the samples, irrespective of the pH or temperature.

The effect of pH and temperature on the biodegradation efficiency of the crude enzyme extract was investigated. The results showed that the biodegradation efficiency of the enzyme was dependent on the pH of the solution and the temperature. The results are displayed in detail in Figures 1-6.The results show that although decolorization varied with temperature and pH, the highest decolorization occurred at 60°Cand a pH of 4 for all three dyes, indicating that this enzyme can withstand high temperatures and low pH.

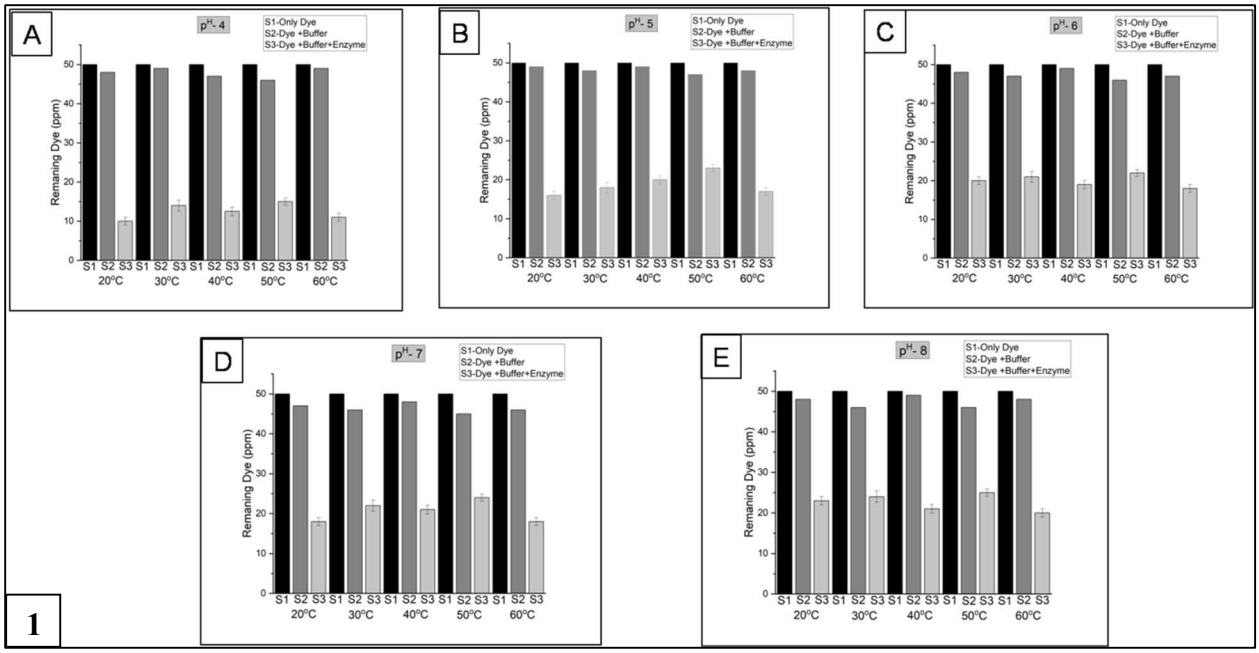

Figure 1A-E shows the effect of pH on the decolorization of RB5at varying temperatures using crude extracellular enzyme isolated from *Anoxybacillus* sp. The decolorization is measured in terms of the initial concentration of the dye, and the results are shown for pH values ranging from 4 to 8. The legend indicates three types of samples: S1, which contains only the pure dye (control); S2, which hasthe dye mixed with buffer; and S3, which includesthe dye, buffer, and crude enzyme. The amount of decolorization is highest in S3, indicating that the crude enzyme can effectively decolorize the dyes. Additionally, the results show that the amount of decolorization varies with pH, with the overall highest decolorization occurring at a pH of 4 and temperature of 20°C.

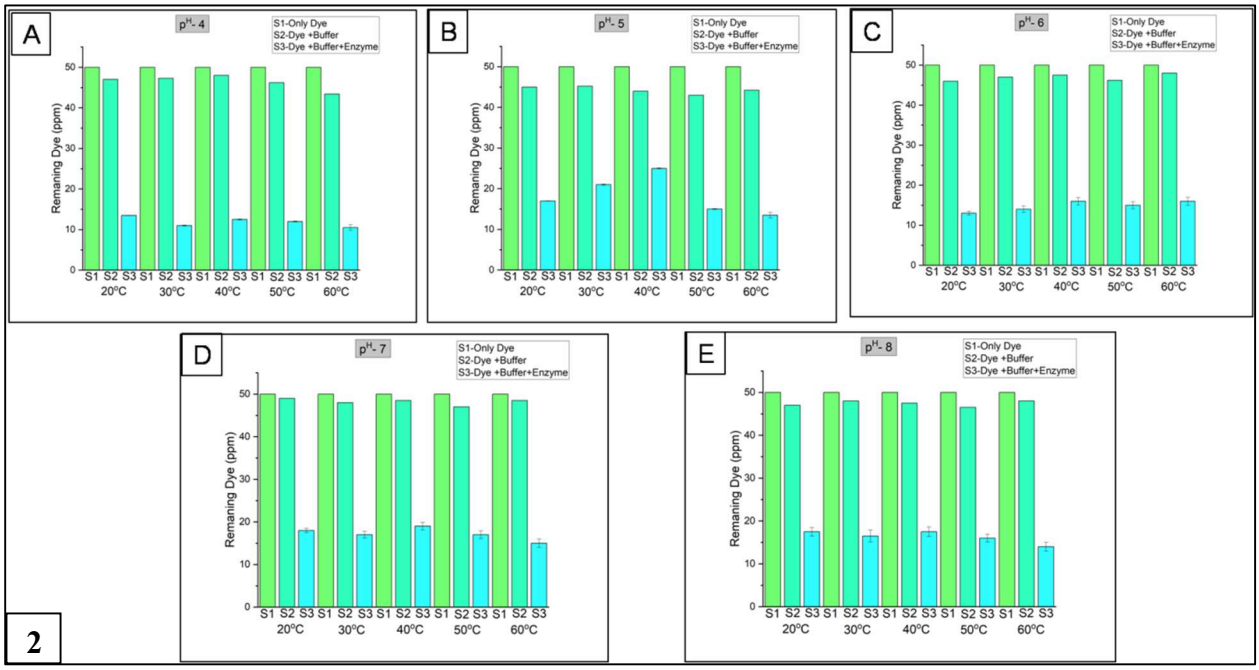

Figure 2A-E shows the effect of pH on the decolorization of Congo Redat varying temperatures using crude extracellular enzyme isolated from *Anoxybacillus* sp. The decolorization is measured in terms of the initial concentration of the dye, and the results are shown for pH values ranging from 4 to 8.The legend indicates three types of samples: S1, which contains only the pure dye (control); S2, which hasthe dye mixed with buffer; and S3, which includesthe dye, buffer, and crude enzyme. The amount of decolorization is highest in S3, indicating that the crude enzyme can effectively decolorize the dyes. Additionally, the results show that the amount of decolorization varies with pH, with the overall highest decolorization occurring at a pH of 4 and temperature of 30°C.

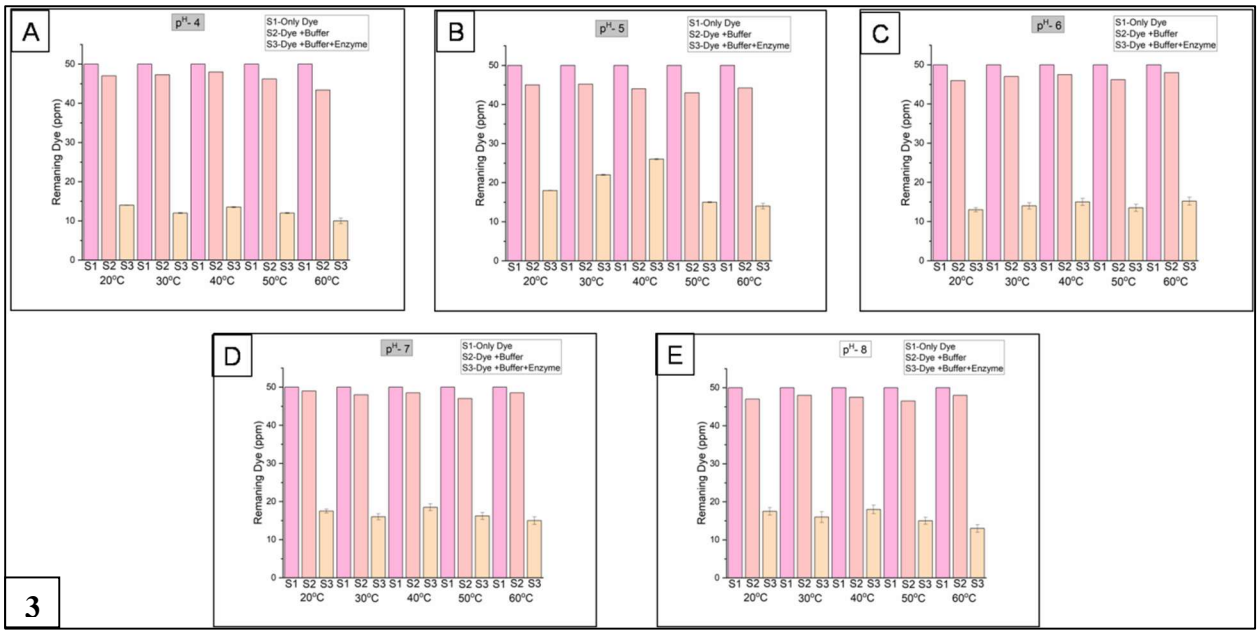

Figure 3A-E shows the effect of pH on the decolorization of Methyl Orange at varying temperatures using crude extracellular enzyme isolated from *Anoxybacillus* sp. The decolorization is measured in terms of the initial concentration of the dye, and the results are shown for pH values ranging from 4 to 8.The legend indicates three types of samples: S1, which contains only the pure dye (control); S2, which hasthe dye mixed with buffer; and S3, which includesthe dye, buffer, and crude enzyme. The amount of decolorization is highest in S3, indicating that the crude enzyme can effectively decolorize the dyes. Additionally, the results show that the amount of decolorization varies with pH, with the overall highest decolorization occurring at a pH of 4 and temperature of 60°C.

Figures 1-3 show the effect of pH on the decolorization of RB5, Congo Red, and Methyl Orange at varying temperatures using crude extracellular enzyme isolated from *Anoxybacillus* sp. The decolorization is measured in terms of the initial concentration of the dye, and the results are shown for pH values ranging from 4 to 8. The legend indicates three types of samples: S1, which contains only the pure dye (control); S2, which hasthe dye mixed with buffer; and S3, which includesthe dye, buffer, and crude enzyme. The amount of decolorization is highest in S3, indicating that the

crude enzyme can effectively decolorize the dyes. Additionally, the results show that the amount of decolorization varies with pH for RB5, Congo Red, and Methyl Orange, with the overall highest decolorization occurring at a pH of 4 and temperature of 20°C, 30°C, and 60°C, respectively.

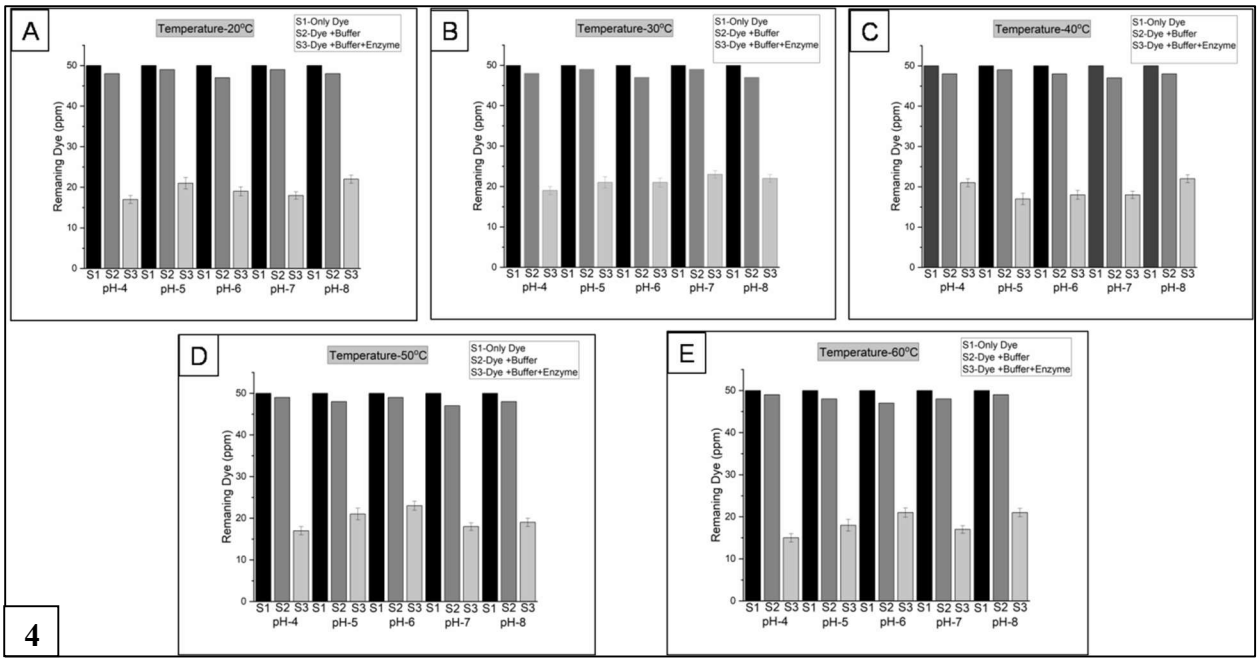

Figure 4A-E shows the effect of temperature on the decolorization of RB 5 at varying pH using crude extracellular enzyme isolated from *Anoxybacillus* sp. The decolorization is measured in terms of the initial concentration of the dye, and the results are shown at different temperatures ranging from 20 to 60°C. The legend indicates three types of samples: S1, which contains only the pure dye (control); S2, which hasthe dye mixed with buffer; and S3, which includesthe dye, buffer, and crude enzyme. The amount of decolorization is highest in S3, indicating that the crude enzyme can effectively decolorize the dyes. Additionally, the results show that the amount of decolorization varies with temperature, with the highest decolorization occurring at 60°C for RB 5 and a pH of 4.

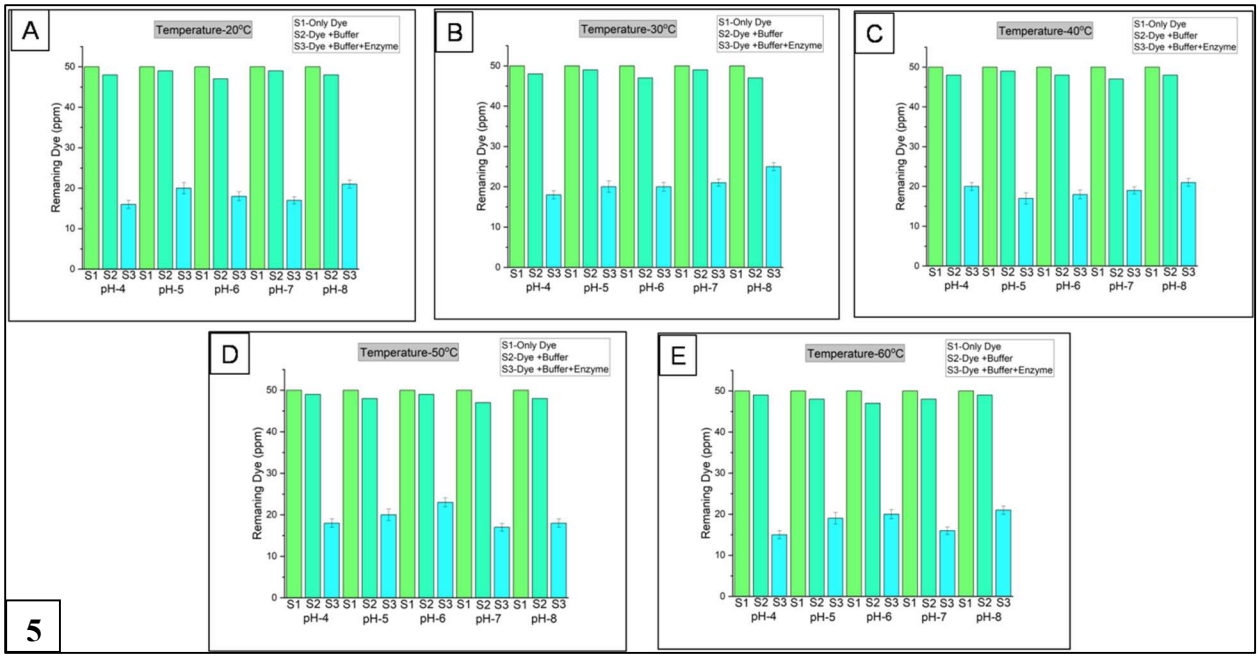

Figure 5A-E shows the effect of temperature on the decolorization of Congo Red at varying pH using crude extracellular enzyme isolated from *Anoxybacillus* sp. The decolorization is measured in terms of the initial concentration of the dye, and the results are shown at different temperatures ranging from 20 to 60℃. The legend indicates three types of samples: S1, which contains only the pure dye (control); S2, which hasthe dye mixed with buffer; and S3, which includesthe dye, buffer, and crude enzyme. The amount of decolorization is highest in S3, indicating that the crude enzyme can effectively decolorize the dyes. Additionally, the results show that the amount of decolorization varies with temperature, with the highest decolorization occurring at 60℃ for Congo Redanda pH of 4.

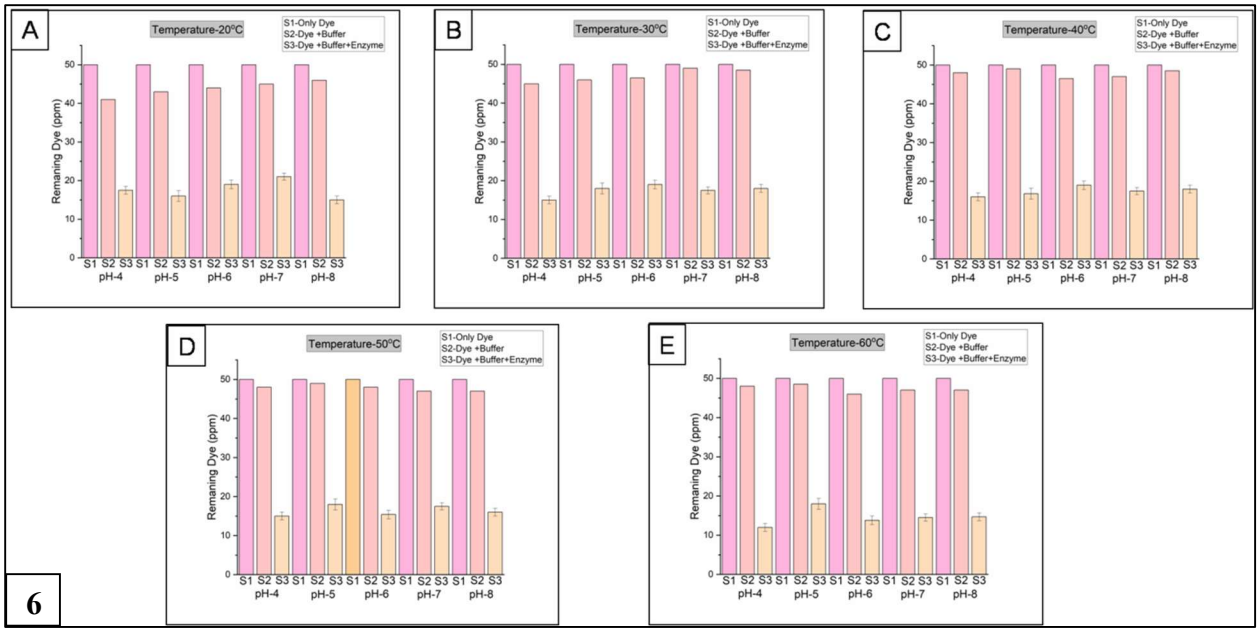

Figure 6A-E shows the effect of temperature on the decolorization of Methyl Orangeat varying pH using crude extracellular enzyme isolated from *Anoxybacillus* sp. The decolorization is measured in terms of the initial concentration of the dye, and the results are shown at different temperatures ranging from 20 to 60℃. The legend indicates three types of samples: S1, which contains only the pure dye (control); S2, which hasthe dye mixed with buffer; and S3, which includesthe dye, buffer, and crude enzyme. The amount of decolorization is highest in S3, indicating that the crude enzyme can effectively decolorize the dyes. Additionally, the results show that the amount of decolorization varies with temperature, with the highest decolorization occurring at 60℃ for Methyl Orangeanda pH of 4.

Figures 4-6 show the effect of temperature on the decolorization of RB5, Congo Red, and Methyl Orange at varying pH using crude extracellular enzyme isolated from *Anoxybacillus* sp. The decolorization is measured in terms of the initial concentration of the dye, and the results are shown at different temperatures ranging from 20 to 60℃. The legend indicates three types of samples: S1, which contains only the pure dye (control); S2, which has the dye mixed with buffer; and S3, which includes the dye, buffer, and crude enzyme. The amount of decolorization is highest in S3, indicating that the crude enzyme can effectively decolorize the dyes.

When we calculated the decolorization efficacy (% decolorization), we found as follows. For RB5, the percentage decolorization at pH 4 and temperature 60℃ was 78%. When we varied the temperature, we found that the percentage decolorization at 60℃ and pH 4 was 70%. For Methyl Orange, the percentage decolorization at pH 4 and temperature 60℃ was 80%. Varying the temperature reduced the efficacy slightly to 76% at 60℃ and pH 4. For Congo Red, the percentage decolorization at pH4 and temperature 60℃ was 70%. When we varied the temperature, the decolorization efficacy increased significantly to 79% at 60℃ and pH 4. When taken across all samples and varying temperatures and pH, we found that the enzyme had its peak efficacy at 60℃ and a pH of 4, indicating that it is thermostable and not denatured by heat or acidity.

Discussion

Textile dye decolorization plays a critical role in environmental preservation due to the detrimental impact of dye presence in water bodies (Thangaraj et al. 2021). These dyes hinder sunlight penetration into aquatic ecosystems, impeding photosynthesis in plants and leading to reduced dissolved oxygen levels, adversely affecting aquatic fauna (Ikram et al. 2022a). Utilizing enzymes for the biodegradation of azo dyes presents a sustainable and promising approach for treating industrial wastewater. Enzymes exhibit cost-effectiveness, environmental friendliness, and high efficiency in azo dye removal (Ikram et al. 2022b). Several studies have been conducted on the biodegradation of azo dyes using crude enzymes; however, not much has been done on the biodegradation capacity of *Anoxybacillus* sp.

This study highlights the efficacy of crude extracellular enzymes obtained from *Anoxybacillus* sp. for azo dye degradation in wastewater. Optimal decolorization was observed at a pH of approximately 4.0 and a temperature of 60°C, with reduced activity noted at pH 6-7, emphasizing the significance of pH and temperature as key factors in achieving high decolorization rates. Notably, the study demonstrates the capacity to enhance crude extracellular enzyme decolorization activity without needing mediators, which are often expensive and toxic, limiting their applicability in biological dye treatment systems. Consequently, the crude extracellular enzyme from *Anoxybacillus sp.* offers a promising eco-friendly and efficient solution for textile dye pollution treatment. In addition, the crude enzyme extract used in this study demonstrated significant versatility. Azo dyes are classified as acidic, basic, sulfur, reactive, disperse, and vat dyes (Vaiano and De Marco 2023).Our enzyme's ability to cause decolorization across acidic, neutral, and basic pH (pH 4-8) signifies its potential to be significantly beneficial in the biodegradation of a wide variety of dyes.

The crude extracellular enzyme isolated from *Anoxybacillus* sp. can break down and degrade certain chemicals, including textile dyes, into smaller and less harmful compounds (Saratale et al. 2011). Our study suggests that crude extracellular enzyme from *Anoxybacillus* sp. can effectively decolorize textile dyes RB5, Methyl Orange, and Congo Red. Our study mirrors the findings of other researchers who have found that crude enzyme extracts are effective in the biodegradation of azo dyes (Bankole et al. 2018; Bozoglu et al. 2013; Vinayak and Singh 2022; Yao et al. 2013). However, our results are even more promising as our crude enzyme extract produced decolorization at just 72 hours. Previous researchers have reported decolorization at 5 days, 10 days, and higher (Tavares et al. 2020).

Overall, using enzymes such as sulfur oxidoreductase (SOR) to decolorize textile dyes is a promising approach to reducing the negative impact of these dyes on the environment (Pal et al. 2022). The results of this study show that the crude extracellular enzyme derived from *Anoxybacillus* sp. has the potential for effective biodegradation of azo dyes. The enzyme degraded the maximum amount of the azo dye in wastewater, as measured by UV-Vis spectroscopy. The crude enzyme analysis confirmed the biodegradation of the azo dye by showing the formation of aromatic amines as by-products. The study also found that the biodegradation efficiency of the

enzyme was dependent on the pH of the solution and the temperature. Mahdi et al. (2022) and Wang et al. (2020) noted that thermophilic bacteria, such as *Anoxybacillus* sp., can grow at high temperatures and produce stable extracellular enzymes. As seen in our study, the crude enzyme extract of *Anoxybacillus* sp. showed evidence of being stable and efficient at high temperatures.

Researchers are striving to find more sustainable and cost-effective approaches to biodegradation. Ikram et al. (2022a, 2022b) investigated the biodegradation of Methyl Red by *Pseudomonas aeruginosa* and Basic Orange 2 by *Escherichia coli* as sustainable and eco-friendly approaches for treating textile wastewater. The authors found that both *P. aeruginosa* and *E.coli* can be utilized as efficient strains for the detoxification and remediation of industrial wastewater containing Methyl Red, Basic Orange 2, and other azo dyes. Chen et al. (2021) conducted toxicity studies on thermophilic *Anoxybacillus* sp. PDR2 and its application potential in bioremediation. The authors demonstrated that this bacterial strain can convert toxic dye into low-toxic metabolites. However, this author's work was limited to the azo dye Direct Black G, unlike our study that considered three azo dyes: RB 5, Congo Red, and Methyl Orange.

Furthermore, isolated crude enzymes present advantages over purified enzymes in terms of cost, stability, and feasibility. Purification methods may compromise enzyme stability, decreasing activity over time (Aziz et al. 2023). In contrast, crude enzymes, due to their lower purification level, exhibit greater robustness and can be utilized for multiple cycles with minimal efficiency loss, reducing the need for frequent enzyme production or replacement. Aziz et al. (2023) found that unpurified enzymes exhibited a greater removal efficiency of dyes than purified enzymes. In addition, large-scale synthesis of purified enzymes can be challenging and costly (Ambatkar and Mukundan 2014). In contrast, crude enzymes can be produced in larger quantities more easily, making them better suited for industrial applications that demand significant enzyme volumes.

Similarly, this study has shown that the crude enzyme extract from *Anoxybacillus* sp. has great potential for the biodegradation of azo dyes. However, further research is warranted to optimize the conditions for purified enzyme biodegradation, encompassing aspects such as purified enzyme concentration, solution pH, and temperature. Additionally, this study exclusively examined the decolorization of three types of azo dyes, calling for further investigations into enzyme biodegradation potential for various kinds of azo dyes. Furthermore, the crude enzyme used in this study is inexpensive but efficient, which is beneficial for developing and low-income countries like India, where this study was conducted. If harnessed, this may present a cost-effective and sustainable method for addressing industrial wastewater treatment in low-income countries.

Conclusion

Enzymatic biodegradation presents a promising and sustainable method for addressing industrial wastewater treatment, particularly in the context of azo dye removal. The study demonstrates the effectiveness of crude extracellular enzymes obtained from *Anoxybacillus sp.* in efficiently degrading azo dyes in wastewater, thus offering a viable, sustainable, and cost-effective approach for industrial wastewater treatment, particularly beneficial for developing and low-income regions. However, additional research is required to fine-tune the conditions for enzyme biodegradation

and explore the full extent of enzyme biodegradation capabilities. The crude enzymatic biodegradation of azo dyes by *Anoxybacillus* sp. shows great potential as a viable substitute for conventional treatment approaches in eliminating azo dyes from industrial effluents. Despite being minimally processed, the crude enzyme demonstrated significant thermostability and decolorization efficacy, achieving up to 80% decolorization in only 72 hours at high temperatures and low pH. This technology provides numerous advantages, including its sustainability, cost-effectiveness, and high efficiency. Further research is necessary to optimize the conditions for the production and application of the enzyme and to evaluate its performance under field conditions.

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