

Effective removal of Direct Orange 26 dye using copper nanoparticles synthesized from Tilapia fish scales

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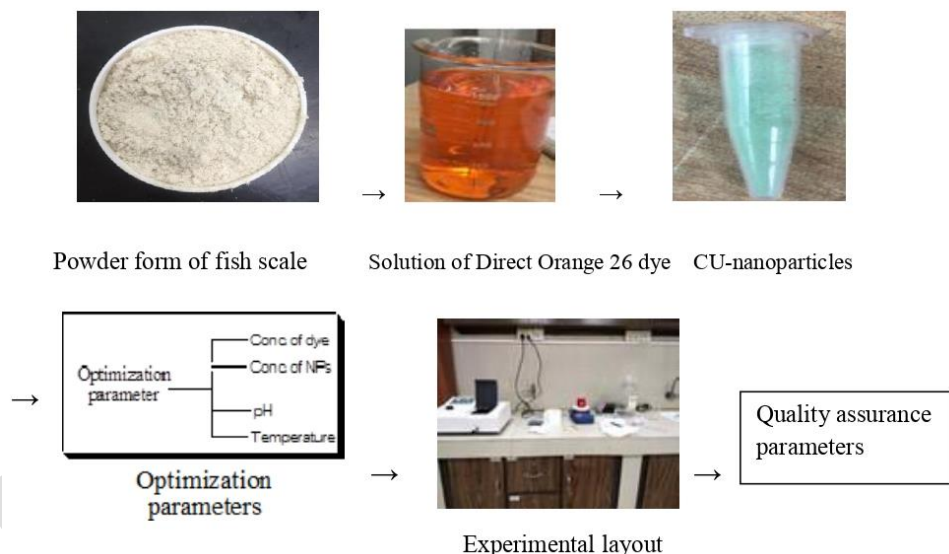
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GRAPHICAL ABSTRACT

Graphical Abstract



Abstract

The performed work reports the manipulation of a collective local surplus solid (fish scales of tilapia) for the synthesis of copper nanoparticles. Copper nanoparticles were synthesized using tilapia fish scales and were characterized using SEM and XRD. The synthesized copper nanoparticles were used for the remediation of direct orange 26 dye. The maximum remediation (90.2%) was noted at 0.01 g/L concentration of dye, 0.001 g/L of Cu-NPs, pH 8, at 40 °C. The

value of TSS for untreated and treated solutions was found to be 0.447 mg/L and 0.40 mg/L, respectively. Similarly, the value of TDS for the untreated solution was found to be 22.35 mg/L while for the treated solution it was 20 mg/L. The percent reduction in COD and TOC were 84.54% and 86.76%, respectively. All data was analyzed statistically. The current study inferred that copper nanoparticles using a tilapia fish scale which is a bio-waste material could be successively used for the detoxification of other toxic dyes as well.

Keywords: Tilapia fish scales, copper nanoparticles; SEM & XRD, direct orange 26 dye remediation, COD & TOC.

1. Introduction

Water is very essential part of life at this universe. Without water life could not exist. As human progress in the field of industrialization, water polluted rapidly. For a healthy life, clean water is very integral (Madhav *et al.*, 2018). Faisalabad is an industrial city, more than 250 different kinds of textile industries are situated in it. Pollution caused by synthetic dyes has become one of the most alarming environmental problems. By rapidly constructing industrial units, pollutants having synthetic dyes are directly or indirectly being discharged into the environment. Elimination of these toxic dyes is very necessary as it destroys the aquatic environment and become a source of many diseases. The natural environment's preservation and improvement is a priority need to be solved. There are physical, chemical, and biological approaches available for this task. Physical procedures are used to transform pollutants from a liquid state to a solid state as a solid sludge (Bilińska *et al.*, 2017). Chemical treatment of industrial effluent may produces more harmful products than their precursors. As a result, these strategies may be less effective for removing notorious dyes. Advanced technologies are invented which are more ecofriendly and are greener ones (Gulzar *et al.*, 2017). Removal of toxic dyes present in textile effluent by nanoparticles is the cost-effective way and a greener approach (Dabas *et al.*, 2019).

Nanotechnology is an emerging, latest, and a multidisciplinary field which deals to construct biomaterials of desired nano shape and size. In 21st century, nanotechnology is one of the essential transformative technologies, and gaining more popularity (Desore *et al.*, 2018). Advances in nanoscience and nanotechnology are expected to not handiest encourage the look

for new phenomena and new thoughts, but can even result in an commercial revolution, so that it will deepen society as a new impetus for financial boom in this century. The applications of nanotechnology are widespread including nano-medicine, sensor, catalysis, drug-delivery, food safety, electronics, separation of materials, solar cells etc. There are two methods for the synthesis of nanoparticles. One is top-down and other one is bottom-up approach (Sahu *et al.*, 2019). The "top-down" methodology is the industrial method that lessens the size of bigger materials. The "bottom up" approach is the construction from individual components (atoms or molecules) by self-assembly, using physical and chemical techniques (Guarnieri *et al.*, 2019).

Tilapia is one of the ancient family of fish. Its zoological name is *Oreochromis niloticus*. In Africa, the Nile tilapia is still vast cultural specie. There are some features of tilapia is their acceptance to underprivileged water quality, and they consume an extensive range of ordinary food animals. Tilapia are seemed to be like sunfish but could easily be judged by an interrupted lateral line, and contain characteristics of the *cichlid* family of fishes (Sarkar *et al.*, 2017). The study of tilapia fish scale gelatin depicts the chemical, structural, and rheological characteristics denote its advantages and uses at industrial scale. The tilapia fish scales comprise of: the gel strength of 233.5g, and gelatin with high amino acid hydroxyproline content (8.10%). This elaborates the gelling capacity of tilapia scale gelatin. The gelatin present in tilapia fish scale has distribution of positive charge, pH range from 6.5 to 8 (Wawrzekiewicz *et al.*, 2017). Hydrolysis of collagen in acidic medium explains that gelatin with polypeptide chain $\alpha 1$ and $\alpha 2$ with β dimer is present. The thermal analysis indicates that the gelatin scales show stability below 200 °C. The tilapia scales gelatin obtained by conventional extraction is very important in food industry and also for the removal of hazards dyes from water (Kishor *et al.*, 2021). The current study was done for the synthesis of copper nanoparticles (Cu-NPs) consuming scales of tilapia fish. Copper nanoparticles were pragmatic to remediate the harmful direct orange 26 dye, applied for the purpose of dyeing in textile sector (Zhang *et al.*, 2018).

2. Materials and methods

All the chemicals and reagents were purchased from Public Scientific Store, Jinnah Colony Faisalabad, Pakistan. Direct orange 26 dye which was used for research analysis also purchased from the same chemical store whose structure is given below in (Figure 1).

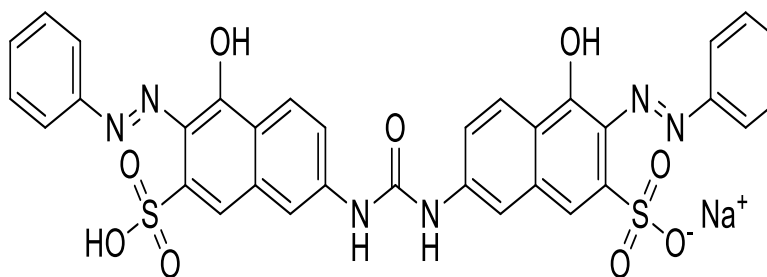


Figure 1. Structure of Direct Orange 26 dye

The current research work has been done on Tilapia fish scales. The fish scale extract was used to synthesize copper nanoparticles. And then, these synthesized copper nanoparticles were employed for the decolorization of Direct Orange 26 dye.

2.1. Sample collection

Fish (*Nile tilapia*) scale were collected from the fishermen's marketplace situated in Jhang Bazar Faisalabad, Pakistan, and washed frequently with distilled water to make it dust & dirt free. The fish scales were then sun desiccated for about 2 days and afterward oven dried at 343 K until the scales became fully dried. The dried scales were grounded to fine powder and sieved to a constant size (100–125 μ m), and then used as a biosorbent without any extra treatment (Haque *et al.*, 2020).

2.2. Preparation of aqueous extract of fish scales

The flask having 500 mL distilled water was taken and 10 g of grind powder of Tilapia fish scales was added in it and heated on hot plate at 70 °C, for 25 min. Hot mixture was shaken at 4000 rpm for 20 minutes. The extract was collected and stored in a refrigerator at 4 °C (Kiran *et al.*, 2020).

2.3. Synthesis of copper nanoparticles (Cu-NPs)

The copper nanoparticles (Cu-NPs) were prepared by following the schematic procedure. 0.5 g of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ was dissolved in 10 g of distilled water, and 50 mL of 10% of fish scale extract was added in to it and pH was maintained to 9. The mixture was frenzied at 100 °C with nonstop shaking and heating for 1 hour until the dark brown color precipitates were formed. These precipitates were filtered, centrifuged, and the acquired precipitates were washed away several times with absolute ethanol and distilled water. Finally, they were dried for two days for further use (Nasar *et al.*, 2019).

2.4. Use of Cu-NPs for Remediation of Direct Orange 26 dye

2.4.1. Preparation of dye solution for scanning of λ_{max}

Various concentrations (0.01%-0.1%) of direct orange 26 dye solution were prepared and were used to scan λ_{max} with ten times dilution. The absorbance value of diluted dye solution was monitored from 400 to 700 nm wavelength range with an interval of 20 nm and checked the wavelength where maximum absorbance took place (Singh *et al.*, 2020).

2.4.2. Experimental procedure

0.01% of dye solution was taken in a reaction vessel and 0.003 g of synthesized Cu-NPs were added into it. This solution is diluted up to 10 times. The reaction vessel was put on a hot plate with magnetic stirrer set at 30 °C. pH of reaction mixture was maintained to 7. Firstly, the absorbance was checked at zero minute was called zero-time absorbance. Then absorbance values were noted at λ_{max} by withdrawing a small aliquot of sample after each 15 minutes. The reaction was run for the time period of 90 minutes. Similar experiments were run with other concentrations (0.02%, 0.03%, 0.04% and 0.05%) of dye solution. Similarly, all other parameters like catalyst dose 0.001-0.005g, pH 4-9, and temperature 30-70 °C, were get optimized by varying one parameter at a time keeping others unchanged (kiran *et al.*, 2020; Ghaffar *et al.*, 2021).

2.4.3. Chemical analysis for optimization of experimental parameters

The rate of the reaction was monitored by determining absorbance at 500 nm via UV-Visible spectrophotometer (Model No MS-4375) with the gap of 15 minutes. Decolorization (%) was intended by the formula given below:

$$\text{Decolorization (\%)} = (I-F)/I \times 100$$

Where I denote the initial absorbance and F stand for the final absorbance of decolorized dye solution.

2.4.4. Mineralization study

The reaction was assessed by different parameters like total dissolve solid (TDS) and total suspended solid (TSS). During reaction different byproducts and metabolites were formed and there TSS and TDS values were found (Kiani *et al.*, 2020). COD is the amount of oxygen spent to chemically oxidize organic water pollutants to inorganic end products. Total organic carbon

(TOC) which utilizes a catalytic oxidation combustion technique at high temperature to convert organic carbon into CO₂. Both treated and untreated samples were assessed for water quality parameters like COD and TOC (Kiran *et al.*, 2020; Kiran *et al.*, 2019; Kiran *et al.*, 2020).

2.4.5. Statistical analysis

All experiments were performed three times. The results were determined by calculating standard error of means (Steel *et al.*, 1997).

3. Results and discussion

3.1. Characterization of synthesized copper nanoparticles by XRD

As depicted in Figure 2, below the X-Ray diffraction was done to characterize synthesized copper nanoparticles, they show the properties of crystalline or amorphous. It also described their particle length (Khani *et al.*, 2018). 14.23° is the angle which shows maximum peak clearly demonstrates the formation of Cu nanoparticles with a polycrystalline cubic phase (Kiran *et al.*, 2020).

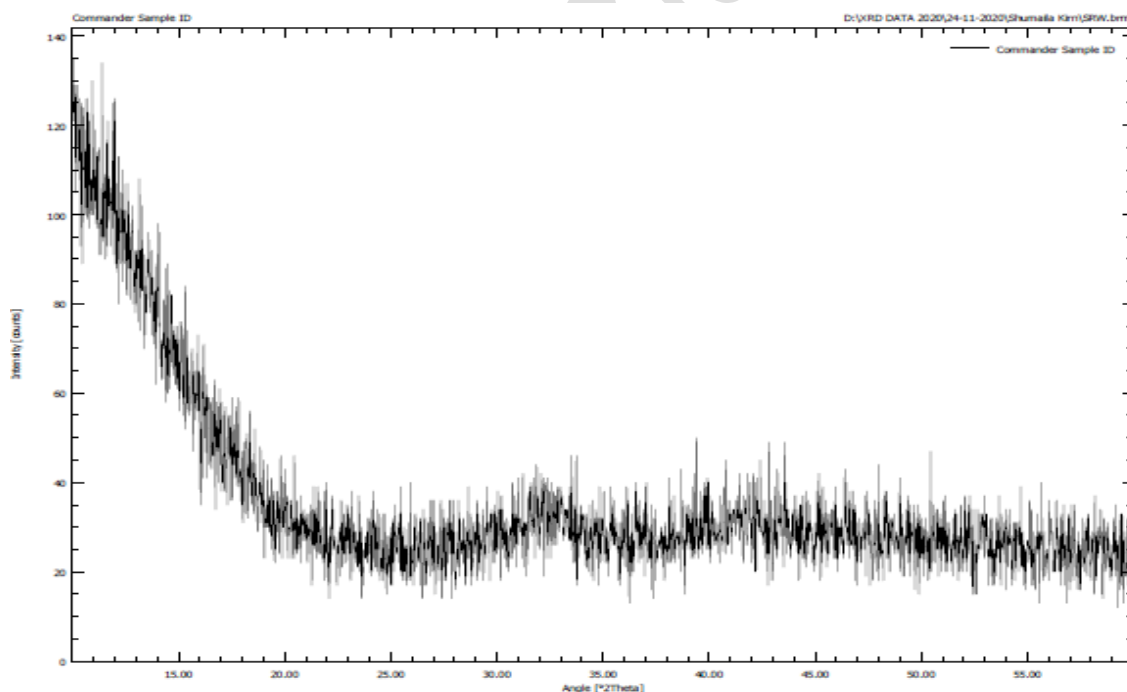


Figure 2. XRD Image of Cu-NPs

3.2. SEM analysis of synthesized Cu nanoparticles

The parameter which was very crucial for the categorization of nanoparticles is their particle size. The sample was placed in glass cuvette and temperature was about 30 °C and the material was processed for 25 seconds. The SEM photographs were designed by means of a scanning electron microscope. The size and shape of developed Cu-NPs was screened by SEM and the results of the images explained spheres such as the structure of Cu nanoparticles found 51-54 nm diameter as shown in Figure 3 (Talebian *et al.*, 2020).

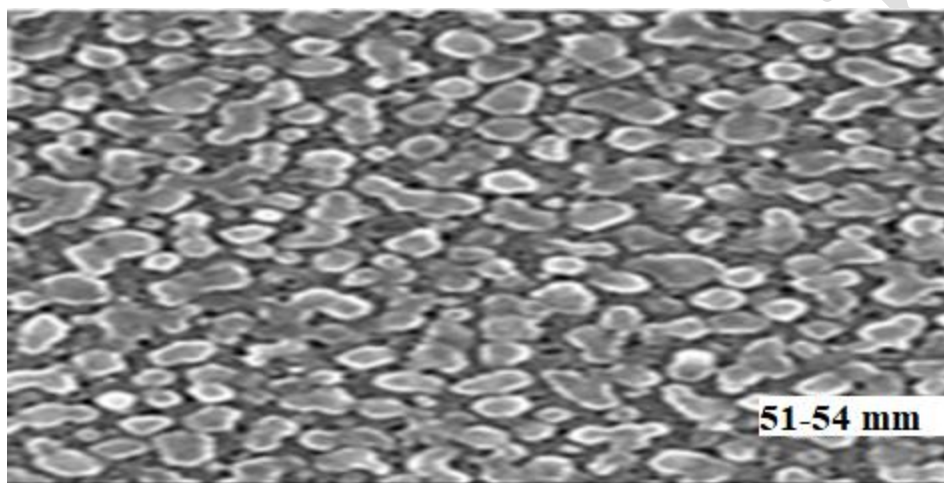


Figure 3. SEM image of Cu-NPs.

3.3. Application of Cu-NPs for remediation of Direct Orange 26 dye

3.3.1. Determination of λ_{max} for Direct Orange 26 dye

From 400 to 700 nm wavelength range in the interval of 20 nm wavelength, absorbance was checked to note the wavelength having maximum absorbance. λ_{max} was found to be 500 nm (Figure 4).

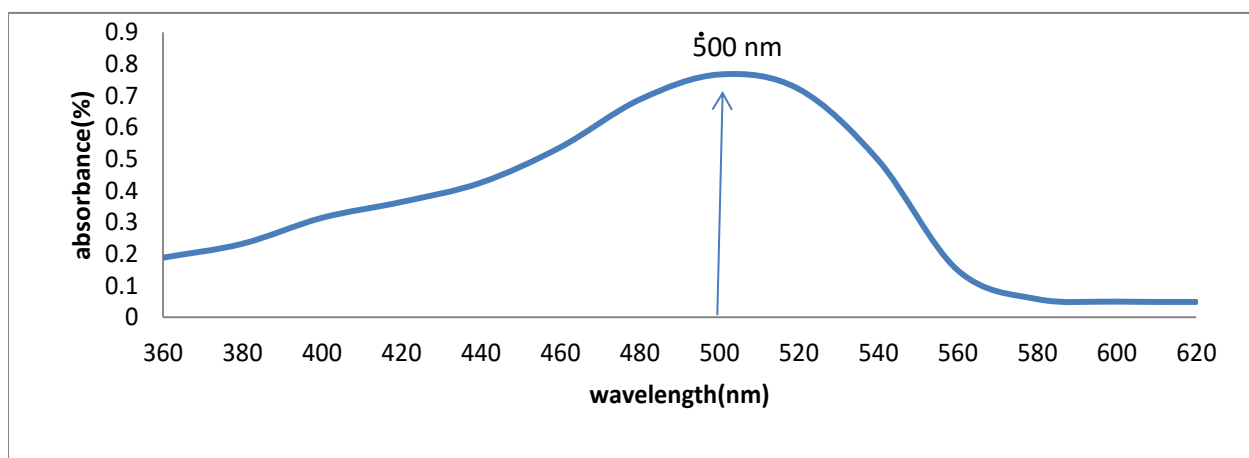


Figure 4. Determination of λ_{\max} for Direct Orange 26 dye.

3.3.2. Optimization conditions for degradation of the Direct Orange 26 dye

The experimental parameters influencing the decolorization rate of Direct Orange 26 dye are; the dye concentration (0.01-0.05%), concentration of the Cu-NPs (0.001-0.005g), pH (4-9), temperature (30-70°C). Each of them was optimized by varying one parameter at a time, keeping others constant.

3.3.2.1. Effect of dye concentration

The quantity of direct orange 26 dye is very compulsory factor. The catalytic decolorization of dye was done at different dye concentrations (0.01-0.05%). As the initial dye concentration was increased, there was an increase in decolorization from 40.5% to 69.5% in 75 minutes. At 0.01% dye concentration, maximum decolorization (69.5%) was occurred (Kiran *et al.*, 2020). After that, as the dye concentration was increased, the %age of decolorization was decreased. (Figure 5a) As the basic amount of dye was enhancing up to saturation point, there were supplementary dye molecules to be cracked up by the catalyst (Kale *et al.*, 2020). Further increases in dye molecules concentration and decolorization of dye may be slowed due to two reasons, firstly the generation of metabolites during the progress of reaction and secondly, lack of larger catalytic sites to catalyze the reaction (Ourique *et al.*, 2018).

3.3.2.2. Effect of copper nanoparticles

Catalyst dose is also play a key role in the decolorization/degradation of dyes. Various experiments were done at different concentrations (0.001g to 0.005g) of nanoparticles to find suitable catalytic dose. It has been noted that as the catalytic dose was increased from 0.001 g to 0.005g, there was maximum percentage of decolorization (78.5%) at 0.001 g of catalytic concentration. After that, there was a gradual decrease in the percentage of decolorization of direct orange 26 dye (Kiran *et al.*, 2020). So 0.001g of copper nanoparticles has been observed suitable catalytic dose for the decolorization of direct orange 26 dye (Figure 5b). According to heterogeneous catalysis, as the concentration of catalytic dose increases, the decolorization/degradation of dye decreases. It is because as the concentration of catalytic dose increase, the amount of active sites at the surface of catalyst also increases. But after a specific amount of catalyst, the more amount of substrate may pollute the sites and consequently hinders the amount of decolorization of dye (Balasooriya *et al.*, 2018). The catalyst activity is saturated at a particular point conferring the maximum availability of its active sites for catalytic action. But above specific amount of catalyst bring about the growth in suspension dirt which fluctuate its catalytic action and consequently there is a decrease in the percentage of decolorization (Sinha *et al.*, 2018; Haque *et al.*, 2020).

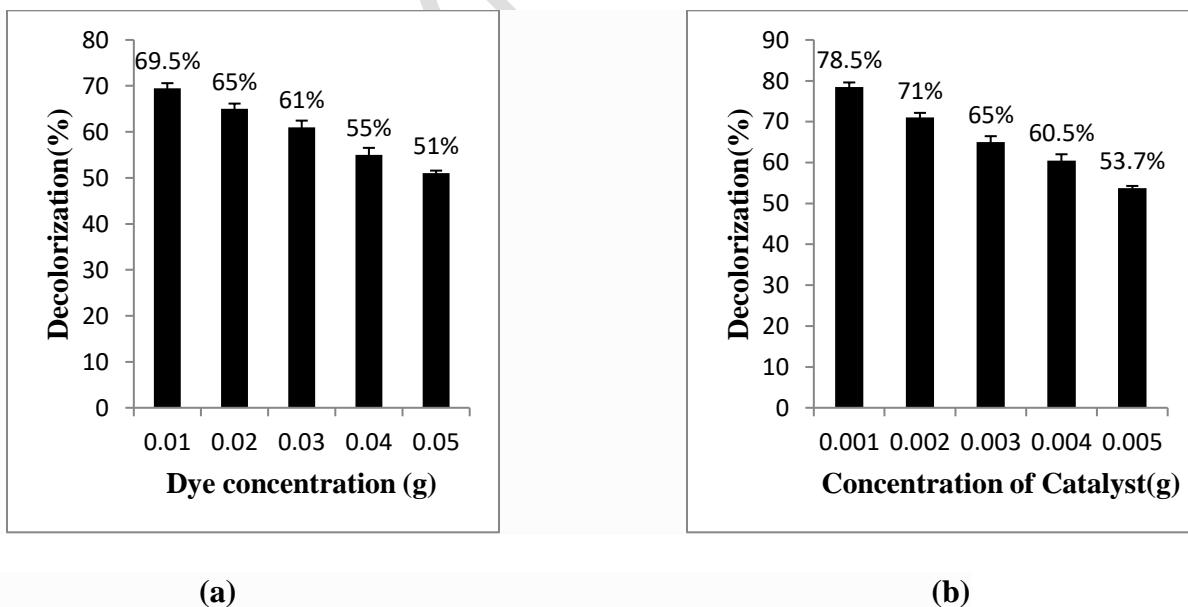


Figure 5. Effect of direct orange 26 dye concentration on its decolorization (a) Effect of different amounts of Cu-NPs on the decolorization of direct orange 26 dye (b).

3.3.2.3. Effect of pH

pH is a crucial parameter. A small variation in pH may results a greater alteration in speed of reaction. So pH optimization is very compulsory (Muddassir *et al.*, 2019). Various levels of pH (4, 5, 6, 7, 8, 9) were selected for its optimization. It was noted that as the pH was increased from 4 to 8, there was an increase in the decolorization from 65% to 82%, respectively (Yusuf *et al.*, 2018). After that it was gradually decrease in percentage decolorization (Figure 6a). On the flip side, at smaller value of pH, H^+ are larger than colliding with the dye molecule which may fluctuate the collaboration of hydroxyl radical with a dye (Ahmed *et al.*, 2019). The solution's pH is a difficult factor which is connected with the ionization state of the nanoparticles surface and with reactants and products (Ahmed *et al.*, 2019). At larger pH, there is a heavy hurdle between negative sites of the dye and nanoparticles anionic exterior which stop the molecules movement from solution to nanoparticles surface. Catalysts perform best when the pH is just right. No significant decolorization of dyes could be achieved for lightly acidic solutions, reaction rates increased at lower pH (Asfaram *et al.*, 2015).

3.3.2.4. Effect of temperature

Temperature plays a key role for the decolorization of dyes. The percentage decolorization was observed at different temperature values ranging from 30 to 70°C. The experiments were done under the optimized parameters such as pH was 8, dose of Cu-NPs 0.001 g/L, amount of direct orange 26 dye was 0.01 g/L. The decolorization was increased up to maximum 90.2% at 40 °C (Verma *et al.*, 2012). After that, the decolorization percentage decreases gradually (Figure 6b). In this process the decolorization capability of direct orange 26 dye decrease as the temperature as further increased. The rise in decolorization (%) with increasing temperature could be attributable to an increase in catalyst binding sites. Decolorization (%) increased as the number of binding sites grew. Furthermore, as the temperature rises, the rate of dye oxidation rises as well, but after a certain temperature, the catalytic effectiveness decreases (Ourique *et al.*, 2018; Kiran *et al.*, 2020).

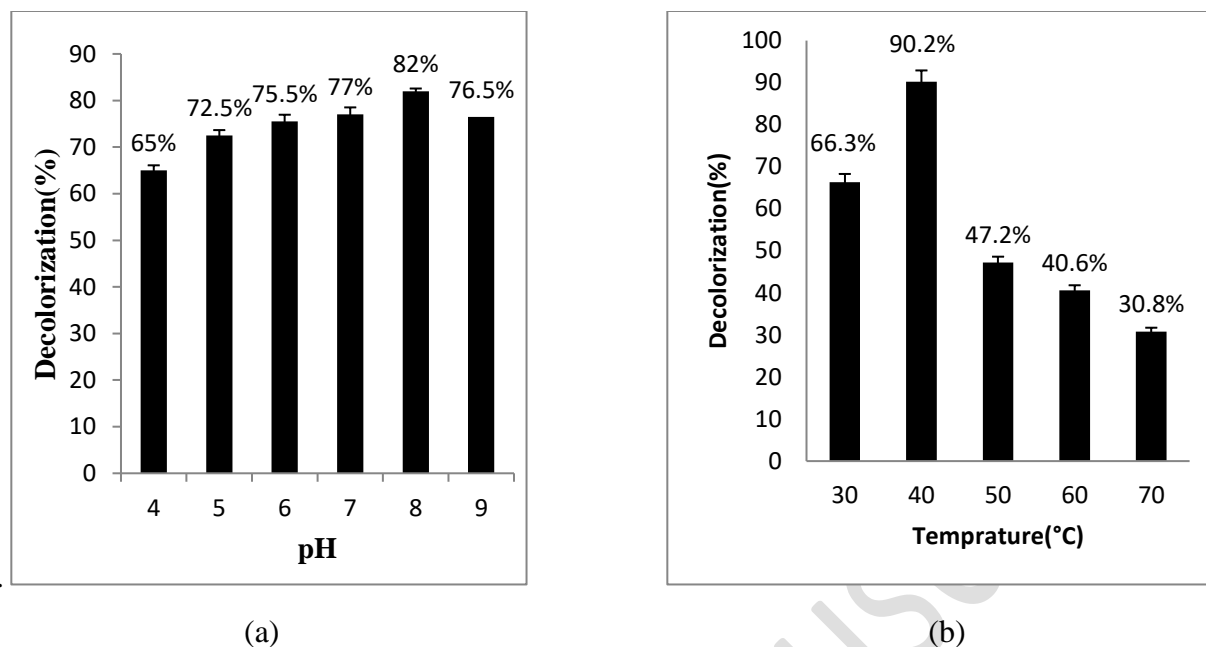


Figure 6. pH effect on direct orange 26 dye decolorization with Cu nanoparticles (a) Temperature effect on decolorization of direct orange 26 dye engaging Cu-NPs (b).

3.3.3. Mineralization study

The reaction was assessed by measurement of TSS and TDS parameters. The value of TSS for untreated and treated dye solutions was found to be 0.447 mg/L and 0.40 mg/L, respectively. Similarly, the value of TDS for untreated solution was found to be 22.35 mg/L while, for treated solution it was 20 mg/L (Yang *et al.*, 2020). As elaborated in figure 7, to check out the mineralization efficiency, the quality checker parameters like total organic carbon (TOC) and chemical oxygen demand (COD) were determined. Percent reduction in COD and TOC values were noted by increasing the reaction contact time from 10 to 70 minutes. The maximum percent reduction in COD and TOC observed were 84.54% and 86.76%, respectively at 50 minute (Parveen *et al.*, 2021). As the time going on the decrease in percentage of COD and TOC elaborate the mineralization under observation. It can be observed that copper nanoparticles not only decolorized but also degraded the direct orange 26 dye which pretended the remediation of dye (Weiss *et al.*, 2020).

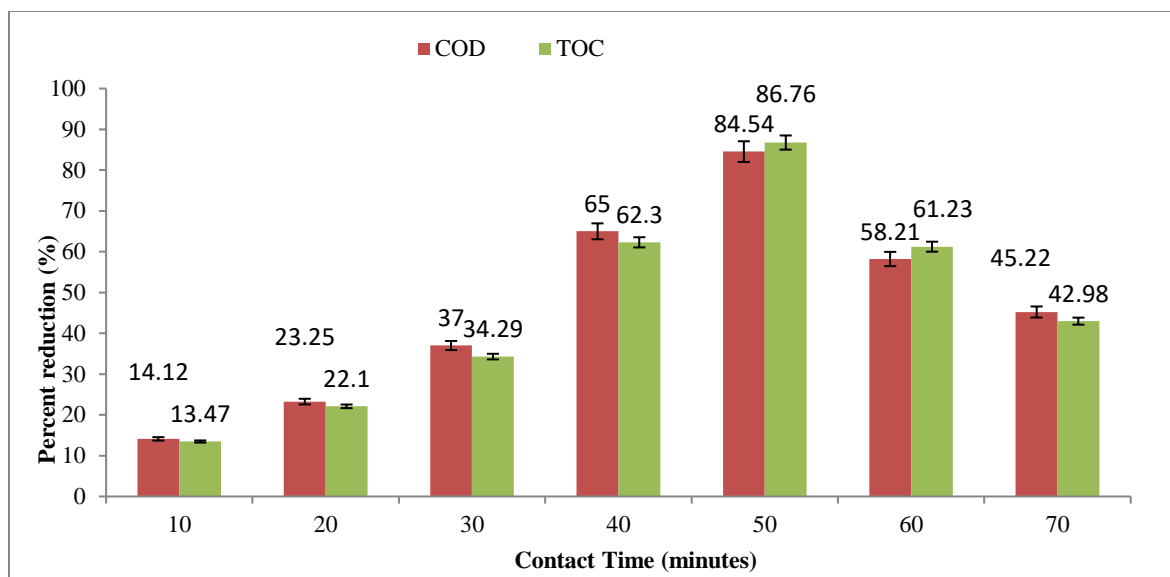


Figure 7. Effect of catalytic process contact time on water quality parameters.

3.3.4. Degradation pathway of direct orange 26 dye

Direct orange 26 dye belongs to the group of azo dyes. Degradation of these dyes takes place in few steps as shown in figure 8. In first step, the azo bond ($N=N$) was broken and phenyl hydrazine, aniline, and intermediate were produced (Mehra *et al.*, 2021). In second step, the dye molecule was further degraded due to breaking of chromophoric group. In third step, molecules are further converted into oxalic acid and others. Finally oxalic acid was degraded into inorganic minerals like CO_2 and H_2O (Jingfei *et al.*, 2016).

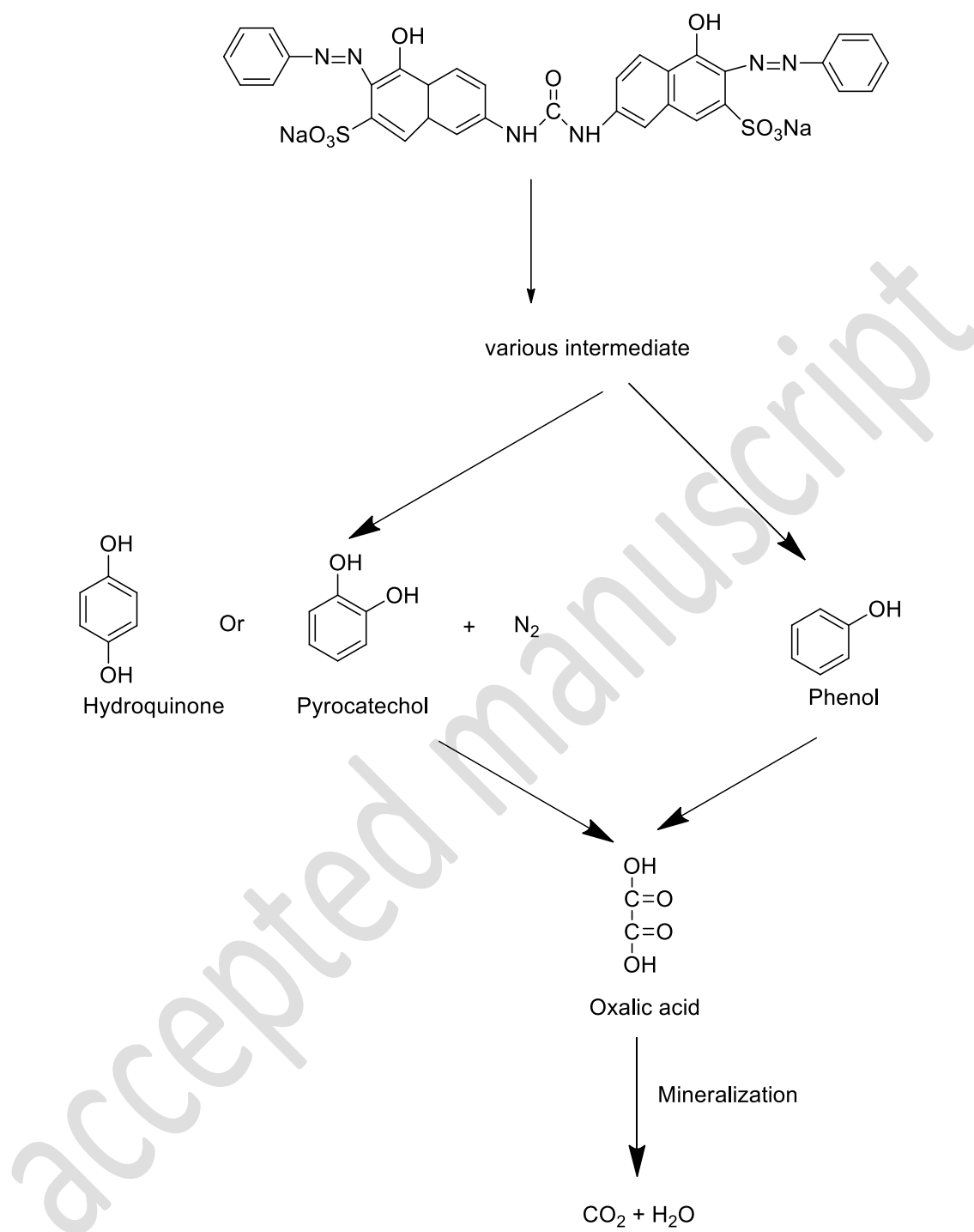


Figure 8: Degradation mechanism of Direct Orange 26 dye

4. Conclusion

The applied method was green, economic and environmental friendly. The study showed that the copper nanoparticles were stable without external use of stabilizing or reducing agent. Domestic waste material of Tilapia fish scales were used for the synthesis of Cu-NPs. This study was done for the degradation of direct orange 26 with the help of Cu nanoparticles prepared by greener method. Under optimized experimental conditions the maximum percentage of remediation was 90.2%, at dye concentration (0.01%), Cu-NPs concentration (0.001 g), pH was 8 at 40 °C. The COD and TOC were determined to be 84.54% and 86.76% respectively. The results of current investigations showed that these prepared Cu nanoparticles successively degraded the direct orange 26 dye into simplest non-toxic product. Using copper nanoparticles, various other notorious water pollution causing dyes can be treated effectively in to simplest end products.

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Disclosure statement

The authors declare no conflict of interest.

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