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Electrochemical and microbial treatment of bromophenol blue dye in aqueous solution

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Abstract

Bromophenol blue (BB) is an important triphenylmethane derivative widely used in research and industry as a dye or colorant for silk, leather, and drugs. BB has a high proclivity for water contamination because of its high solubility and stability, causing damage to living organisms even in low quantities. This study assessed the feasibility of electrochemical and biological for the decolorization of BB using graphite-copper electrode and indigenous microorganisms, respectively. It was found that effectiveness of electrochemical treatment improves with higher electrolysis time, current density, and electrolyte concentration, drops with rising pH, and shows a nonlinear pattern with temperature. The microbial strains identified as *Acinetobacter baumannii*, *Serratia marcescens*, *Aspergillus niger*, *Aspergillus flavus*, *Bacillus megaterium*, *Rhizopus stolonifer*, and *Bacillus subtilis* performed poorly, irrespective of the dye/mineral salt ratio. The electrochemical technique was much more effective for decolorizing bromophenol blue dye-contaminated water. The computational results showed clearly that bromine atoms are the most susceptible sites for attack by oxidizing species and, thus, the onset of BB-decolorization.

Keywords

Wastewater treatment; dye removal efficiency; quantum chemical calculations; graphite electrodes; dye pollution; environmental remediation

Introduction

Dyes are an important class of pollutants, and once they reach the water bodies, remediation becomes difficult due to their synthetic origins and complicated molecular structures. Dye-containing wastewater are steadily generated by textile industries [1].

Bromophenol blue dye (BB) is an important triphenylmethane derivative widely used in research and industry as a dye/colorant for silk, leather, and drugs, as well as a laboratory indicator, biological stain, and color maker to keep track of the polyacrylamide gel electrophoresis processes and

agarose gel electrophoresis. BB has a high proclivity for water contamination because of its high solubility and stability, causing damage to living organisms even in low quantities [2]. The molecular structure of BB is shown in Figure 1.

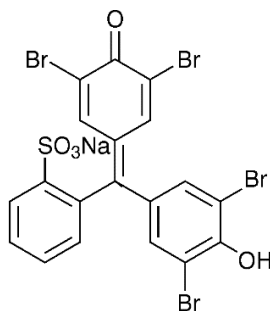


Figure 1. Molecular structure of bromophenol blue dye

Since BB is labeled highly toxic and persistent, several remediation technologies have been investigated for its removal from aqueous systems. These technologies include adsorption [2], photo-catalysis [3], electrochemical treatment [4], and biotic and biological treatment [5]. It is interesting to note that over 50 % of the literature on BB decolorization is focused on photo-catalysis and other light-induced methods. Although photocatalytic oxidation is a fast and effective method for dye decolorization, it has the disadvantage of being expensive and requires harsh operating conditions, elaborate setup, and management procedures [6]. Electrochemical degradation has been employed in wastewater treatment in recent years because of its high efficiency, low cost, and low formation of secondary pollutants [7].

Rong *et al.* [8] have experimented with the electrochemical degradation of BB using boron-doped diamond (BDD) electrodes with positive results, while Zhang *et al.* [9] assessed the role of sulfate electrolytes on electrochemical decolorization of BB in BDD anode cells Zhao *et al.* [10] reported 96.9 % electrochemical decolorization efficiency for BB achieved using PbO₂-ZrO₂ composite electrodes. Composite electrodes like BDD and PbO₂-ZrO₂ represent the new trend in contaminant electrochemical degradation technologies due to their superior degradation performance and remarkable current efficiencies [11]. Conversely, such composite electrode materials often fail to serve as durable electrodes in sizeable electrooxidation operations and are prohibitively expensive for industrial-scale applications [12]. Hence, dimensionally durable and low-cost electrodes are still required for long-term, large-scale applications [13].

Many studies have been conducted on using carbon-based electrode materials for the effective electrochemical decolorization of dyes in aqueous solutions. Zakaria *et al.* [14] investigated the electrochemical oxidation of reactive orange 16 using carbon-based electrodes from biomass, obtaining a decolorization efficiency of up to 98.5 %. With graphite material used as electrodes, Oguzie *et al.* [15] investigated the electrochemical decolorization of dispersed blue-1 dye in an aqueous solution achieving a decolorization efficiency of 96 %. Luan *et al.* [16] employed carbon nanotubes for electrochemical oxidation of methylene blue and the reduction of carbon dioxide to obtain valuable products. Goren *et al.* [17] studied the electrochemical removal of methylene blue and reported up to 99.9 % removal efficiency using flexible graphite electrodes with operation costs as low as 0.012 \$/m³.

Microbial treatment (bioremediation), involving the breakdown of complex organic substances into less toxic smaller molecules by means of living organisms, is another inexpensive and benign option for color removal from aqueous systems. Microorganisms are able to degrade synthetic dyes

via contaminant adsorption on the microbial biomass or biodegradation *via* enzyme production [18]. Potadar *et al.* [19] successfully employed bacterial isolates of *Pseudomonas sp.* and *Salmonella sp.* from water samples for the treatment of effluents contaminated with BB. Chmelova and Ondrejovic [20] employed lacase produced by *Ceriporiopsis subvermispota* to degrade BB, achieving decolorization efficiency of more than 89.8% with a mediator, and 49.8 % without a mediator. Singh and Singh [21] successfully degraded BB with *Aspergillus flavus* and *Trichoderma harzianum* fungi.

This study assesses the feasibility of electrochemical and biological methods for the decolorization of BB using graphite/copper electrodes and indigenous microorganisms, respectively. The influence of different process variables on the efficiency of the electrochemical and biological processes is assessed. Quantum chemical computations are also carried out to identify the sites where decolorization is initiated on the dye molecule.

Experimental

Chemicals and reagents

Bromophenol blue dye, MacConkey agar, Sabouraud Dextrose Agar and nutrient agar were obtained from Aldrich, while other reagents like sulphuric acid and sodium hydroxide were obtained from Sinopharm, China. All reagents were used as sourced without additional purification.

Preparation of dye-polluted soil

One gram of BB dye was diluted in 1 L of distilled water to make 1000 ppm of BB dye solution. A soil sample was collected on a farm beside the SOPs extension building at the Federal University of Technology, Owerri, using a soil auger. The soil auger was inserted 30 cm below the soil, which was used to obtain 4.5 kg of soil. One liter of 1000 ppm of BB dye solution was then mixed with the soil sample.

Preparation of culture media

MacConkey agar: Fifty-two grams of Mac Conkey agar powder were dispersed in one liter of deionized water. Allowed to soak for 10 minutes before swirling to mix and sterilizing by autoclaving at 121 °C for 15 minutes. After cooling to 47 °C, the solution was mixed and poured into Petri dishes.

Sabouraud dextrose agar: sixty-five grams of the powder was dissolved in 1000 ml distilled water. The mixture was boiled to dissolve the medium completely at 121 °C for 15 min and cooled to 45 to 50 °C. Then it was properly mixed and dispersed.

Nutrient agar: twenty-eight grams of nutrient agar powder was dissolved in 1 L of distilled water, swirled to mix properly and dissolved completely. The solution was sterilized by autoclaving at 121 °C for 15 min. The liquid was then poured into a Petri dish and allowed to solidify.

Preparation of mineral salt

The media composition used for this study is a mineral salt medium (broth) with the following composition: 1.0 g/L NH₄NO₃; 0.2 g/L MnSO₄·7H₂O; 1.0 g/L K₂HPO₄; 0.1 g/L CaCl₂·2H₂O; 0.2 g/L KCl; 1.0 g/L FeSO₄·6H₂O; 0.1 g/L ZnSO₄·7H₂O; 1.0 g/L MnSO₄ and 1.0 g/L yeast extract which was dissolved in 1 L of sterile water and autoclaved at 121 °C for 15 minutes.

Microorganism isolation and identification

A ten-fold serial dilution of BB dye-polluted soil was carried out as described by Cheesebrough [22]. This dilution process was carried out to a diluent factor of 10 (D10). Thereafter, samples from diluent D5, D7, and D9 were inoculated on the three different media in triplicate. The micro-

organisms were then subcultured by streaking on a sterile Nutrient agar plate to obtain pure culture for microbial identification and characterization following a series of biochemical tests as described in [22] and [23].

Microbial decolorization experiments

Mineral salt medium (50 mL) and 100 mL of 50 mg/L BB dye solution were mixed in a conical flask. A loopful of each isolated microbial strain was separately inoculated into the mixture. The setup was allowed to stand for 9 weeks. About 4 ml of the mixture is aspirated once a week, centrifuged at 4000 rpm for 15 min, and sent for analysis. The procedure was followed for 100 mL of mineral salt and 50 mL of BB dye solution.

Electrochemical decolorization experiments

Electrochemical decolorization experiments for BB were conducted in a 500 mL capacity electrolytic cell equipped with a magnetic stirrer. An anode of graphite with dimensions of 60×25×2.5 mm and a cathode of copper of the same size were used. Both electrodes were connected to a digital DC power supply (model MCH-K305D) with specific voltage and current settings. The electrolytic bath was prepared by mixing 490 mL of 30 mg/L of BB dye solution with 10 mL of supporting electrolyte. The pH of the solution was adjusted using 1 M H₂SO₄ or 0.1 M NaOH as needed. A pH meter (PHS-3C) was used to determine the desired pH values (3, 7, 9, 11). The mixed BB solution was then added to the electrolytic cell, and the electrodes were submerged in the solution. The electrolysis process was initiated by applying the programmed current density and 25 V settings on the DC power supply. During the electrolysis process, samples were collected at 10-minute intervals for up to 70 minutes. The collected samples were then subjected to analysis using spectrophotometry. The experimental variables considered in the study included the initial solution pH (3, 7, 9, 11), supporting electrolyte type (KCl and NaCl) of concentrations (1.00, 0.50, 0.10, 0.05 M), temperature (301, 311, 321 and 331 K), and current density (15 - 75 mA/cm²). Each experiment was conducted in triplicate to ensure statistical reliability.

Analytical procedure

The solutions aspirated from the electrolysis cell and microbial treatment sample bottles at intervals were monitored using a Shimadzu UV-3600 Plus UV-Vis-NIR spectrophotometer, over a wavelength range of 200-800 nm. The peak at the maximum wavelength ($\lambda_{\text{max}} = 590 \text{ nm}$) was especially monitored for dye solutions before and after electrolysis as an indicator for BB decolorization. The full wavelength scan enabled visualization of other absorption peaks associated with BB degradation intermediates. The BB removal efficiency was calculated as follows:

$$DE = \frac{A_0 - A_t}{A_0} 100 \quad (1)$$

where A_0 is the initial absorbance of the BB solution before electrolysis and A_t is the absorbance of BB at the given time t .

Quantum chemical computations

Quantum chemical computations were undertaken using the DMol3 package of Material Studio software (BIOVIA Materials Studio Academic Research Suite (Product No: 5CB-LUR)).

Results and discussion

Identification of microorganism

Biochemical tests and morphological characterization of different isolates were used in identifying the microbial strains. The code and associated names of the isolated strains shown in Table 1 reveal a total of 7 distinct isolates from soil contaminated with BB dye. This implies that the dye served as a source of nutrients for the seven isolates but was toxic to other species in the soil ecosystem, due to the known toxicity of triphenylmethane dyes. A similar result has been reported by other authors, for instance, *Serratia marcescens* [24], *Aspergillus sp* [25], *Bacillus subtilis* [26], *Bacillus megaterium* [27], *Rhizopus stolonifera* [28], *Eubacterium sp* [18], *Acinetobacter baumaniu* [29] have all been reported to show some efficacy in decolorization of textile dyes.

Table 1. Isolated microbial strains from the polluted soil sample

Sample	Name of strain	Organism type
BB1	<i>Acinetobacter baumaniu</i>	Bacteria
BB2	<i>Serratia marcescens</i>	Bacteria
BB3	<i>Bacillus subtilis</i>	Bacteria
BB4	<i>Bacillus megaterium</i>	Bacteria
BB5	<i>Aspergillus niger</i>	Fungus
BB6	<i>Aspergillus flavus</i>	Fungus
BB7	<i>Rhizopus stolonifera</i>	Fungus

Microbial decolorization of BB

To investigate the BB decolorization efficiency of the isolated micro-organisms, 50 ppm of the dye-contaminated water with the mineral broth was inoculated with the different identified strains, respectively. The results are shown in Figure 2.

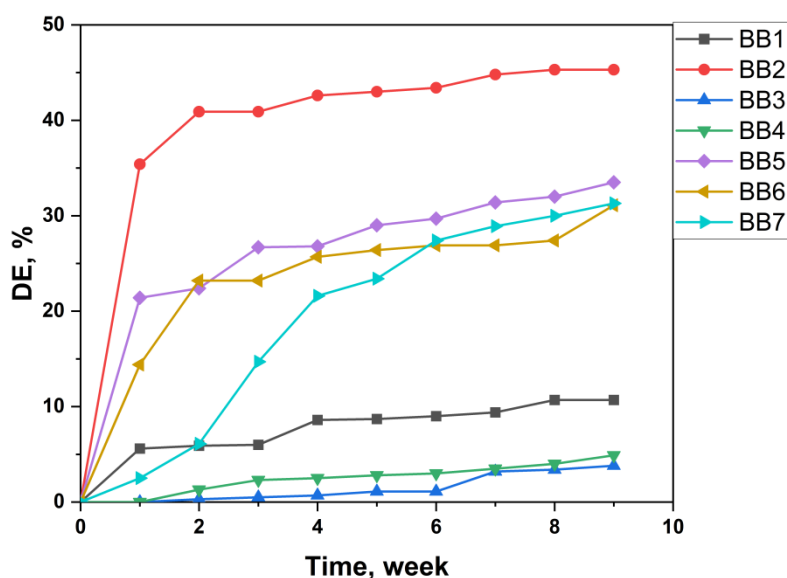


Figure 2. Biodecolorization efficiency of BB by different microbial strains: BB1 - *Acinetobacter baumaniu*; BB2 - *Serratia marcescens*; BB3 - *Bacillus subtilis*; BB4 - *Bacillus megaterium*; BB5 - *Aspergillus niger*; BB6 - *Aspergillus flavus*; BB7 - *Rhizopus stolonifer*

The identified microbial strains were found to have varying abilities for decolorizing BB dye since the efficacy of microbial decolorization depends on the adaptability and activity of individual microorganisms. Figure 2 shows that *Serratia marcescens* (BB2) achieved the highest decolorizing

effect for all time intervals, while *Bacillus subtilis* (BB3) achieved the lowest. Decolorization efficiency for all strains increased with time. Dye decolorization can occur in two ways, *i.e.*, by biosorption on microbial biomass or bio-decolorization of dyes by microbial cells [30]. Dye adsorption can occur on both growing/living and dead microbial cells. Biosorption preserves the original structure of the dyes, as the dye does not undergo degradation. On the other hand, biodegradation destroys the original dye structure, degrading it into smaller molecules and moieties, occasionally accomplishing complete mineralization into CO₂ and H₂O.

Effect of dye and mineral salt on microbial decolorization of bromophenol blue

The effect of dye and mineral salt on the decolorization efficiency of BB was investigated by performing decolorization experiments using two different volume ratios of dye and broth solution. Figure 3 shows the effect of dye and mineral salt volume ratio on microbial decolorization of BB.

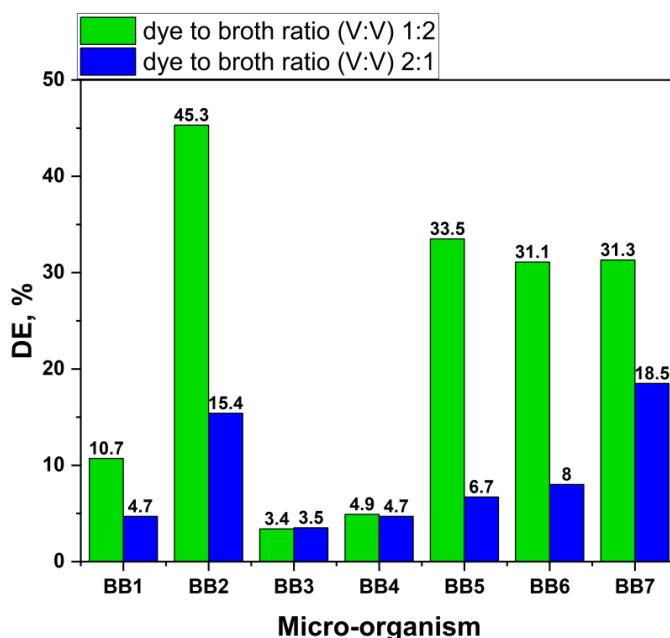


Figure 3. Effect of dye/mineral salt volume ratio on biodecolorization efficiency: BB1 - *Acinetobacter baumannii*; BB2 - *Serratia marcescens*; BB3 - *Bacillus subtilis*; BB4 - *Bacillus megaterium*; BB5 - *Aspergillus niger*; BB6 - *Aspergillus flavus*; BB7 - *Rhizopus stolonifer*

Figure 3 shows that *Acinetobacter baumannii* (BB1), *Serratia marcescens* (BB2), *Aspergillus niger* (BB5), *Aspergillus flavus* (BB6), and *Rhizopus stolonifer* (BB7) achieved higher decolorization efficiency for 1:2 dye to broth volume ratio, while *Bacillus subtilis* (BB3) and *Bacillus megaterium* (BB4) performed poorly irrespective of the dye/mineral salt ratio. The presence of dangerous levels of dyes may affect the growth of microorganisms, *i.e.*, the ability to decolorize colors. The dye class governs its structural properties and influences how much a dye gets decolorized. The amount of nitrogen and glucose in the medium can also influence dye decolorization by altering the capacity of bacteria to create enzymes. This is consistent with our findings that ratio 1:2 outperforms ratio 2:1.

Electrochemical decolorization of BB

Electrochemical techniques have been found effective for the treatment of organic pollutants in wastewater. Since organic pollutants do not readily yield electroactive species that can respond to an applied electric field, electrolyte salts that readily dissociate into electroactive species are added to the solution to enhance electrical conductivity. Other factors influencing electrochemical treatment techniques include solution pH, initial contaminant concentration, applied current and temperature.

Effect of supporting electrolyte on electrochemical decolorization of BB

Supporting electrolytes, especially chlorides, play a crucial role in electrolysis processes, especially those involving organic species, which are very poorly electroactive and have low conductivity. Therefore, supporting electrolytes are added to enhance ionic conductivity and decrease solution resistance. Experiments were carried out to ascertain how different concentrations of KCl and NaCl applied as supporting electrolytes, influence the electrochemical decolorization of BB. The obtained results are illustrated in Figure 4a for KCl and Figure 4b for NaCl. Parameters held constant for the experimental runs include BB concentration, temperature, current density, and voltage.

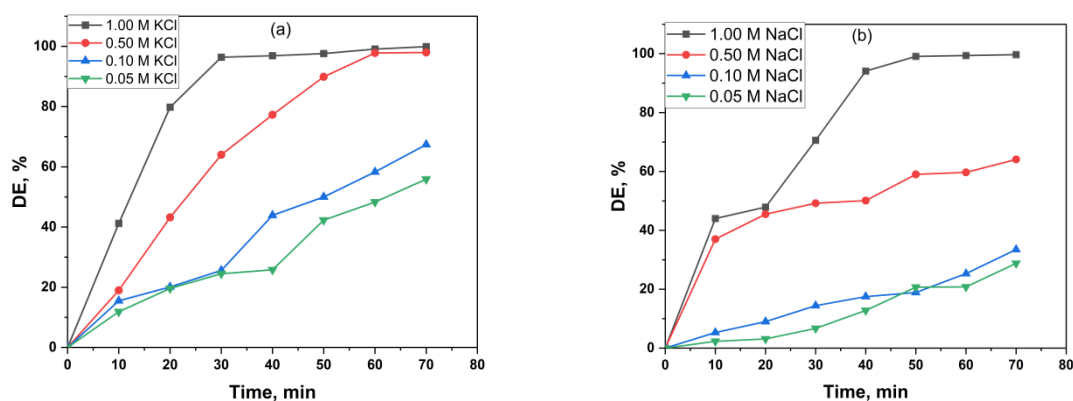


Figure 4. Effect of KCl (a) and NaCl (b) as supporting electrolytes on BB decolorization efficiency (dye concentration = 30 mg/L, pH 7, temperature = 301 K, voltage = 25 V, current density = 75 mA/cm²)

Both plots show that decolorization efficiency increased steadily with supporting electrolyte concentration, attaining optimum values of 99.9 and 99.7 % in 1 M KCl and 1 M NaCl, respectively, suggesting that both salts exerted identical effects. However, the results also show that the KCl system attained optimal efficiency within 30 min, whereas the NaCl took 50 min. This implies that KCl is a better-supporting electrolyte, yielding more rapid decolorization. Moreover, values of 55.9 and 28.8 % were recorded at the lowest electrolyte concentration (0.05 M) for KCl and NaCl, respectively. Also, 0.5 M KCl achieved close to optimal decolorization, albeit at a much longer time interval (60 min). This is because KCl has a larger electronegativity difference, K atoms are larger than Na atoms, and K is more electropositive than Na. As a result, KCl is a more ionic substance than NaCl. It should be noted that too high a concentration of the supporting electrolyte may create adverse consequences. For instance, high concentrations of chloride salts could promote the formation of unwanted organochlorides [31], as well as high concentrations of hypochlorous acid, which can promote corrosion of some electrode materials. So, one has to make a trade-off between speed and efficiency in deploying higher or lower salt concentrations.

Effect of initial pH of BB solution on electrochemical decolorization efficiency

The efficacy of the electrochemical decolorization processes is significantly influenced by electrolyte solution pH. The pH dependence of BB decolorization efficiency as a function of time is plotted in Figure 5.

The variation of decolorization efficiency with initial solution pH presents an interesting trend within the time interval of the experiments. Although generally, the decolorization efficiency decreased as pH increased from acidic *via* neutral to alkaline over the available time interval, almost optimum efficiency is attained at each pH value, at different time intervals (30 min at pH 3, 40 min at pH 7, 50 min at pH 9 and 70 min at pH 11). This implies that just the solution pH influences mostly the kinetics of the decolorization process.

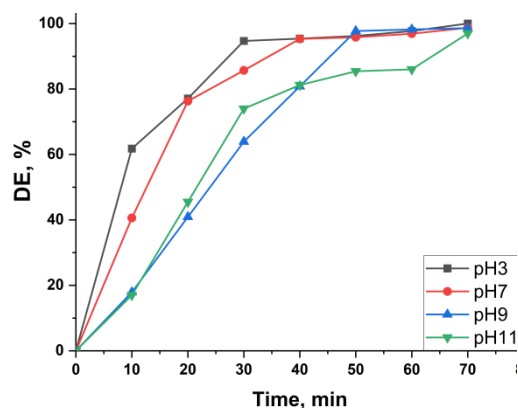


Figure 5. Effect of initial pH of electrolyte solution on BB decolorization efficiency (dye concentration = 30 mg/L, current density = 75 mA/cm², temperature = 301 K, voltage = 25 V, electrolyte = 1 M KCl)

pH impacts various properties, including chemical states of solution species and solvent molecules, electrostatic interactions at the electrode/solution interphase, the kind of active oxidizing species generated during the reaction, etc., making thus any interpretation difficult. The processes of oxygen generation and oxidative destruction of organic molecules on the anode are always competing in the anodic oxidation system. The speed of oxygen production at the anode surface rises as the pH of the solution increases. This reduces the oxygen evolution potential, delays organic pollutant transport to the anode, and so reduces the rate of organic matter oxidation at the anode electrode [4]. On the other hand, when the environment is acidic, the electrochemical conditions for organic pollutant degradation at the anode surface get better as the oxygen evolution process slows [32]. Additionally, the conductivity of the solution diminishes under alkaline circumstances due to a lack of appropriate supporting electrolytes, which further reduces the effectiveness of organic pollutant decomposition [33]. This supports higher degradation efficiency in the acidic than in an alkaline medium [34]. Therefore, pH 3 was found as the ideal condition for BB decolorization. In order to avoid strong acid conditions, further experiments were performed at pH 7, which according to Figures 1 and 5 give acceptable decolorization efficiency results.

Effect of current density on electrochemical decolorization of BB

The correlation between BB degrading efficiency and current densities from 15 to 75 mA/cm² is shown in Figure 6.

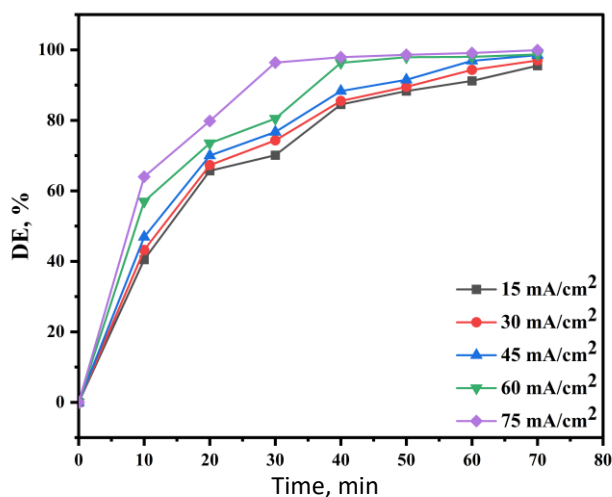


Figure 6. Effect of current density on decolorization efficiency of BB (dye concentration = 30 mg/L, temperature = 301 K, voltage = 25 V, electrolyte = 1 M KCl, pH 7)

The plots follow the expected trend of increased decolorization efficiency with an increase of current density over the entire time interval, optimal efficiency and kinetics attained at the highest studied current density of 75 mA/cm². Again, very appreciable decolorization efficiencies were obtained at all current densities, albeit at different rates.

Current density has been recognized as one of the most essential parameters controlling the anodic oxidation process, capable of regulating the rate of electrochemical degradation. Increasing the current density over a constant period improves the efficiency of electrochemical degradation by boosting electron flow between the anode and cathode and increasing electrochemical transformations as well as contaminant oxidation at the anode [34].

Using a higher current density in the electrochemical cell accelerates electron transfer kinetics at the interface and prevents the accumulation of electrolysis intermediates that could deactivate electroactive sites on the anode. A much higher current density, on the other hand, may increase side reactions associated with oxygen generation at the anode electrode, which interferes with organic matter oxidation and, eventually, reduces the efficacy of pollutant oxidation. It is as well noteworthy that high current densities will increase power requirements and overall operational costs [1].

Effect of temperature on electrochemical decolorization of BB

Experiments were undertaken at 301 to 331 K to determine the optimal temperature for the decolorization of BB solutions. The obtained results are shown in Figure 7. Although there is clear evidence that the decolorization rate increased with an increase in temperature, the trend was established over the entire time interval following the order 301 < 331 < 321 < 311 K.

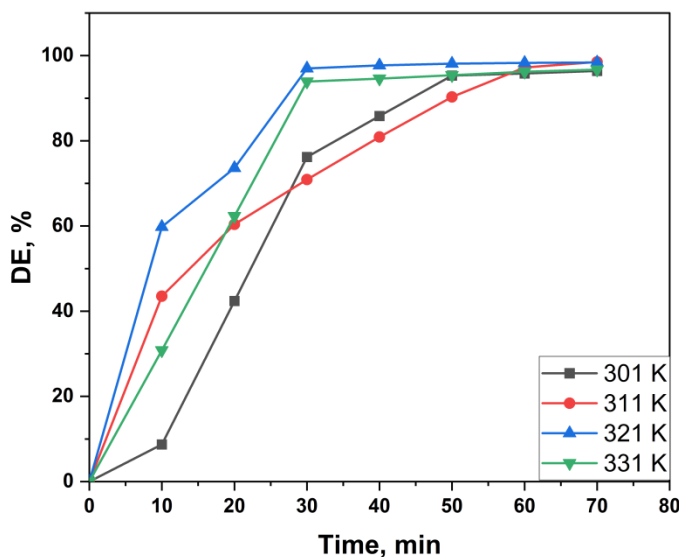


Figure 7. Effect of temperature on decolorization efficiency of BB

(dye concentration = 30 mg/L, voltage = 25 V, electrolyte = 1 M KCl, pH 7, current density = 75 mA/cm²)

Nonetheless, decolorization at all temperatures ultimately attained near-optimal efficiency but at different reaction times. Superior decolorization kinetics is attained with the system at 321 K, attaining higher optimal efficiency values after 30 min, while the 331 K system attained slightly lower values at the same time. Such inconsistencies in the influence of temperature on the electrochemical degradation of organic contaminants arise because high temperatures can either enhance the oxidizing power of the highly oxidizing species that degrade the contaminants or hinder the generation of oxidizing chloride species or both [35]. The prevailing effect would depend on the nature of the organic compound and the environment under study.

Electrochemical degradation profile of BB

Time-dependent full wavelength scans were obtained for BB solutions as electrolysis progressed. By monitoring the intensity of the characteristic absorption peak of the BB chromophore at $\lambda_{\max} = 590$ nm, as a function of electrolysis time, it is possible to ascertain the efficacy of the electrochemical decolorization process using graphite and copper electrodes. Figure 8 illustrates the full wavelength absorption spectra for BB after electrolysis for time intervals ranging from 0 to 70 min. The plots show that the intensity of the BB absorption peak at 590 nm was markedly diminished after electrolysis treatment for 10 min and decreased steadily thereafter until it completely disappeared after about 30 min. This is clear evidence that the electrolysis cell employing a copper cathode and a graphite anode very effectively degrades BB and achieves complete mineralization of the dye contaminant. The appearance of new peaks between 380 nm and 430 nm indicates the presence of less conjugated reaction intermediates, which are subsequently degraded with time. As observed by Jović *et al.* [35], the features of BB breakdown on anodes include quick mineralization and little aromatic intermediate buildup.

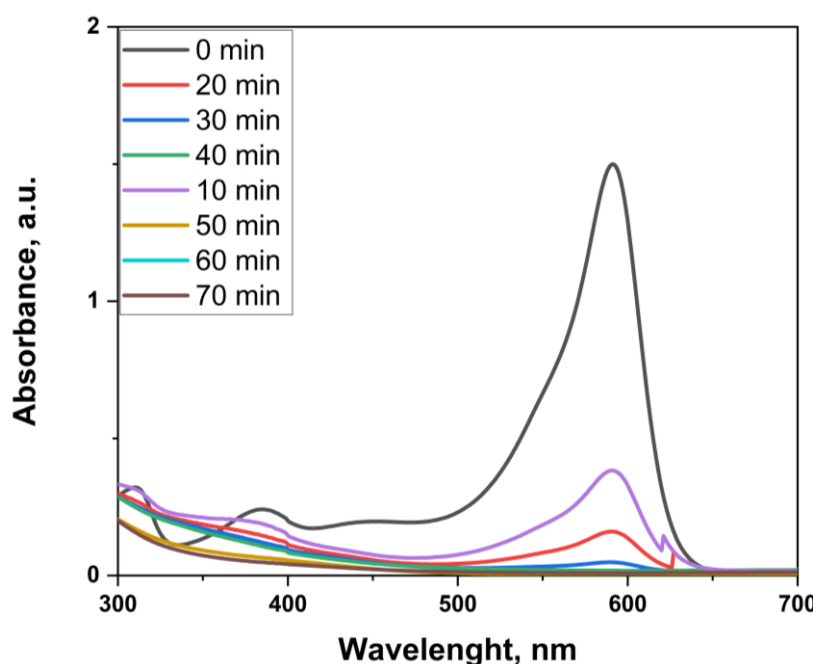


Figure 8. Full wavelength UV-Visible absorption spectra showing time-dependent electrochemical decolorization behavior of BB (dye concentration = 30 mg/L, voltage = 25 V, electrolyte = 1 M KCl, pH 7, current density = 75 mA/cm²)

Electrochemical decolorization kinetics

The electrochemical decolorization process is often facilitated by *in-situ* generation of strongly oxidizing species formed *via* electrochemical transformation of the supporting electrolyte(s) added to enhance solution conductivity. For instance, chloride ions from chloride salts undergo reactions with hydroxyl radicals in the vicinity of the anode to form hypochlorous acid (HOCl), and hypochlorite ions (OCl⁻) that act as the main oxidizing agent in oxidative degradation [36], equations (2) to (4):



The generated oxidizing species then initiate the attack at certain susceptible sites, mostly electron-rich centers on the contaminant structure. The most convenient way to monitor dye

decolorization kinetics is to track the gradual decrease in dye concentration as a function of time. Figure 9 shows the kinetic plots for BB decolorization at $T = 301$ K, derived from the first-order kinetic equation (5):

$$\ln(A/A_0) = -kt \tag{5}$$

where A_0 = initial BB absorbance, A = BB absorbance at time t , and k = rate constant for electrochemical decolorization for BB, which is determined from the slope of the kinetic plot.

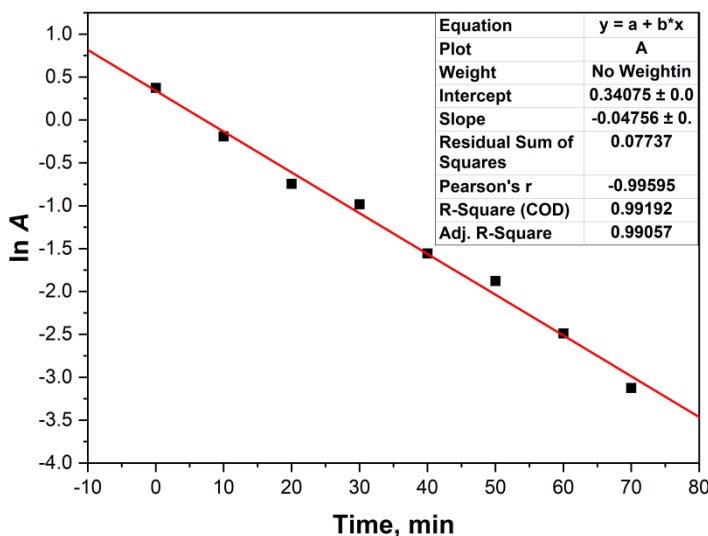


Figure 9. First-order kinetic plot for electrochemical decolorization of BB

The good linear fit of the plot obtained means that the electrochemical decolorization of BB from our experiments obeyed the first-order kinetics law.

Energy consumption

By determining the amount of electrical energy (kWh) used during the electrochemical decolorization of BB, the energy consumption related to this process may be determined using the equations (6) and (7):

$$E = \frac{Pt}{V_{sol}} \tag{6}$$

where:

$$P = Iv \tag{7}$$

P / W is the electrochemical cell electrical output, t / h is the electrolysis time, V_{sol} / dm^3 is the amount of dye solution electrolyzed, I / A is the current, and v / V is voltage. Table 2 highlights the process of energy consumption for BB decolorization, calculated at different treatment times: 30, 50, and 70 min and currents of 1 to 5 A, which makes it possible to calculate how much energy is used to treat each cubic meter of BB contaminated water, according to the revised Nigeria electricity tariff, which has recently been increased to 0.09 USD/kWh.

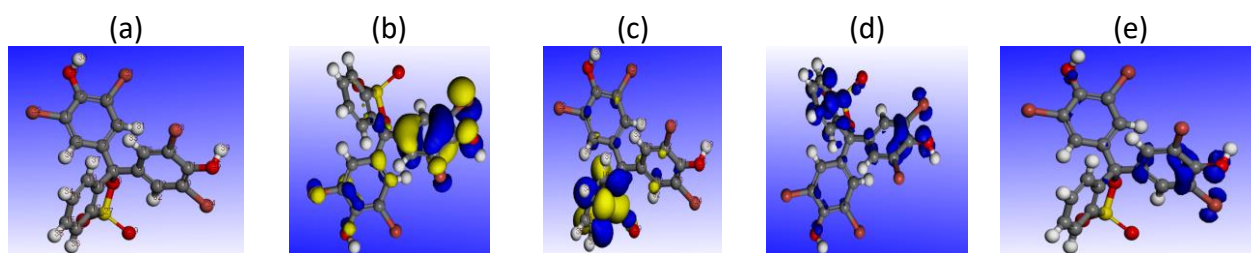
For a given current value, energy consumption increased with treatment time and for a given treatment time, energy consumption increased with current. So, achieving optimal efficiency of 99 %, at the peak current value (5 A) after 30 mins will consume 125 kWh/m³ of energy, with a total cost of 11.53 USD. Whereas achieving similar decolorization efficiency at 4 A takes 50 min and will consume 166.67 kWh/m³ at a total cost of 15.32 USD.

Table 2. Calculated energy requirements for electrochemical treatment of BB solution at different currents and treatment time at 301 K

Current, A	Voltage, V	Time, min	Energy consumption, kWh m ⁻³
1	25	0.50	25.00
		0.83	41.67
		1.17	58.33
2	25	0.50	50.00
		0.83	83.33
		1.17	116.67
3	25	0.50	75.00
		0.83	125.00
		1.17	175.00
4	25	0.50	100.00
		0.83	166.67
		1.17	233.33
5	25	0.50	125.00
		0.83	208.33
		1.17	291.67

Electrochemical decolorization initiation mechanism

To determine the mechanism of BB decolorization, at least theoretically, DFT computations were carried out to model the electronic structure and local reactivity of the dye molecule to enable the identification of the active sites for the oxidative attack. The Hirshfeld population analysis [37], the Perdew-Wang (PW) local correlation, limited spin polarization on the DND basis set, and the DMol3 software (Materials Studio) were all employed in the computations. The detailed computational protocol is reported elsewhere [15]. Figure 10 depicts the optimized structure, highest occupied molecular orbital (HOMO), Fukui function, and lowest unoccupied molecular orbital (LUMO) of BB.

**Figure 10.** Electronic properties of BB: (a) optimized structure; (b) HOMO; (c) LUMO; (d) Fukui F^0 ; (e) Fukui F^-

The corresponding quantum chemical parameters are given in Table 3. The HOMO regions, which measure the propensity of the molecule to donate electrons, lie around the bromine-bearing nuclei, while the electrophilic Fukui functions depicting the sites most prone to attack by electron-seeking species coincide with the locations of the electronegative bromine atoms.

The electronic structures can be further assessed by considering basic molecular reactivity indicators within the framework of the hard and soft acids and bases (HSAB) theory [37]. The computed quantum chemical parameters like the HOMO and LUMO energies, chemical hardness (or softness), HOMO/LUMO energy gap, *etc.*, are key structure-activity indicators relating electronic structure to chemical reactivity.

Table 3. Calculated quantum chemical parameters for BB

Chemical parameters	
$E_{\text{HOMO}}^*/\text{eV}$	-6.099
$E_{\text{LUMO}}^*/\text{eV}$	-2.546
Energy gap ($\Delta E_{\text{gap}} = (E_{\text{LUMO}} - E_{\text{HOMO}})$), eV	3.553
Electrochemical potential ($\mu = -X$), eV	-4.323
Ionization potential (I), eV	6.099
Electron affinity (AE), eV	2.546
Electronegativity ($X = (I + A) / 2$), eV	4.323
Chemical hardness ($\eta = (I - A) / 2$), eV	1.777
Chemical softness ($S = 1 / \eta$), eV	0.563
Electrophilicity ($\omega = \mu^2 / 2\eta$), eV	5.258
ΔE back donation ($-\eta / 4$), eV	-0.444

Table 4 presents Fukui indices (F^i) for electrophilic (F^-) and radical (F^0) attack, which allows the determination of the reactivity order of the various functional groups in the BB molecule with respect to strongly oxidizing species and radicals. The results show clearly that the bromine atoms (especially Br (23) and Br (24)) represent the most susceptible sites for attack by both radical (F^0) and electrophilic (F^-) species and represent the most probable sites for attack and onset of decolorization of BB.

Table 4. Fukui indices for the electrophilic and radical attack on BB

Atom No	F^-	F^0	Atom No	F^-	F^0
C (1)	0.023	0.017	Br (21)	0.027	0.024
C (2)	0.012	0.012	Br (22)	0.068	0.044
C (3)	0.015	0.008	Br (23)	0.082	0.050
C (4)	0.015	0.012	Br (24)	0.147	0.082
C (5)	0.012	0.012	O (25)	0.091	0.050
C (6)	0.022	0.016	O (26)	0.007	0.015
C (7)	0.005	0.004	S (27)	0.005	0.024
C (8)	0.052	0.027	O (28)	0.011	0.030
C (9)	0.031	0.022	O (29)	0.009	0.028
C (10)	0.051	0.030	H (30)	0.008	0.008
C (11)	0.065	0.038	H (31)	0.010	0.008
C (12)	0.036	0.023	H (32)	0.017	0.012
C (13)	0.037	0.021	H (33)	0.019	0.012
C (14)	0.008	0.062	H (34)	0.005	0.027
C (15)	0.011	0.036	H (35)	0.005	0.019
C (16)	0.008	0.052	H (36)	0.005	0.024
C (17)	0.005	0.039	H (37)	0.005	0.018
C (18)	0.001	0.019	H (38)	0.009	0.006
C (19)	0.005	0.036	H (39)	0.027	0.015
O (20)	0.030	0.019			

In order to obtain some insights into the nature and stabilities of BB degradation intermediates reported by [38] and [39], further quantum chemical computations were performed to model the electronic structures of the molecules and determine their theoretical enthalpies of formation. This is because intermediate products with more negative ΔH_f values would be more stable and less prone to undergo side reactions, and *vice versa*. Figure 11 shows the electronic structures of BB and the major degradation intermediates and corresponding enthalpies of formation.

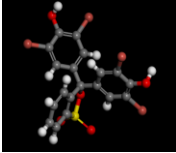
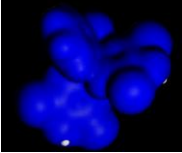
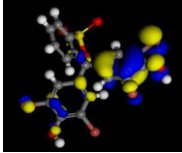
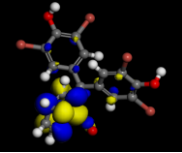
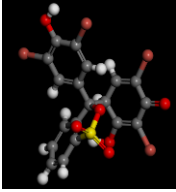
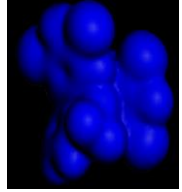
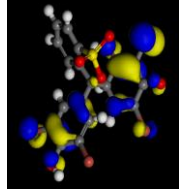
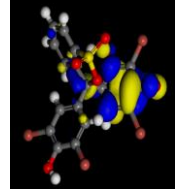

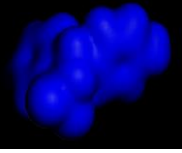
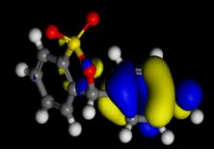
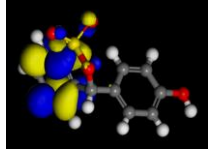
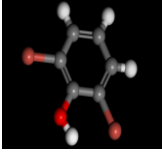
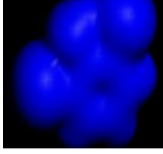
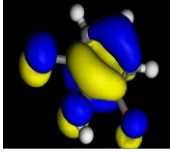
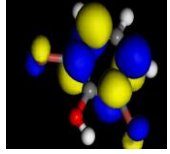
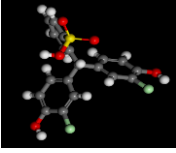
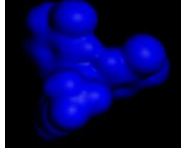
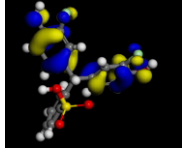
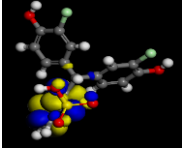
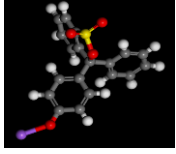
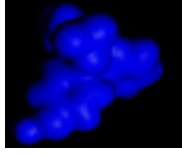

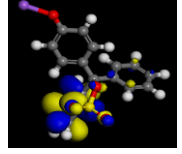
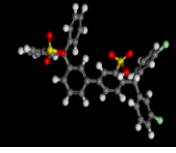
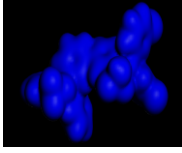


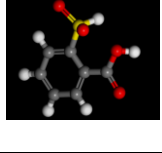
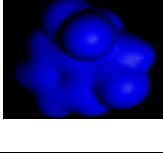
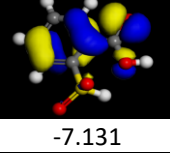
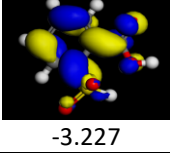
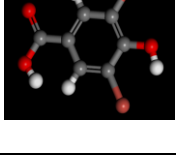
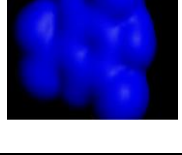
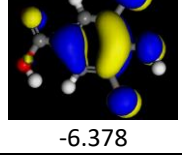
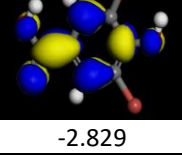
Comp.	Molecular structure	Total electron density	HOMO orbital E_{HOMO} / eV	LUMO orbital E_{LUMO} / eV	Δ_f / J mol ⁻¹
BB			 -6.099	 -2.546	-110.3
BB-1			 -6.097	 -3.556	-137.0
BB-2			 -5.893	 -2.324	-130.0
BB-3			 -6.023	 -1.852	-16.2
BB-4			 -5.750	 -2.471	-182.1
BB-5			 -4.916	 -2.494	-128.0
BB-6			 -6.126	 -2.734	-124.3
BB-7			 -7.131	 -3.227	-181.5
BB--8			 -6.378	 -2.829	-111.1

Figure 11. Electronic structures of BB and major degradation intermediates and corresponding enthalpies of formation

The enthalpy of the formation of a compound can be used as a measure of its relative stability compared to its constituent elements. If the enthalpy value is negative, then it is an exothermic compound and its elements could theoretically be made to react and form the compound. Conversely, a positive enthalpy of formation value indicates that the compound is relatively unstable compared to its elements and that its formation would require energy. However, this does not necessarily mean that the elements cannot be made to react and form the compound directly.

Conclusion

In this study, electrochemical and microbial techniques were employed in the decolorization of bromophenol blue dye-contaminated water. Seven microorganisms that could decolor BB dye, especially at lower dye concentrations, were isolated. The electrochemical technique using graphite anode and copper cathode was found more effective for the decolorization of BB that followed the first-order reaction kinetics. A decolorization efficiency of 99 % was reached after 30 min with increasing current density up to 75 A/cm². The highest decolorization efficiency was observed at pH 3, and lowest at pH 11. Decolorization efficiency showed a nonlinear relationship with temperature rise. The computational results showed clearly that bromine atoms are the most susceptible sites for attack by oxidizing species and, thus, the onset of BB decolorization.

Conflict of interest: All authors declare no conflict of interest.

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