Abstract: We report the preparation of a series of spacer-incorporated, tetra-amino cobalt (II) phthalocyanine (CoPc)-immobilized bacterial cellulose (BC) functional nanocomposites (CoPc@s-BC). Four kinds of flexible spacers with different lengths—diethylenetriamine (DT), triethylenetetramine (TT), tetrathylenepentamine (TP) and pentaethylenehexamine (PH)—were covalently attached onto pre-oxidized BC for the synthesis of the spacer-attached BC, and the attached spacers’ contents were carefully quantified. Using glutaraldehyde as a cross-linker, the CoPc catalyst was covalently immobilized onto the spacer-attached BC, and the immobilization steps were optimized by monitoring both the residual spacer contents and the resulting immobilized CoPc. All of the functionalization processes were characterized and confirmed by X-ray photoelectron spectroscopy (XPS). The series of spacer-incorporated, CoPc-immobilized BC nanocomposites, CoPc@s-BC, were used for the decoloration of dye wastewater. Both the adsorption capacity and adsorption rate were increased after the incorporation of spacers. When H₂O₂ was employed as an oxidant, dye molecules were catalytically oxidized with these nanocomposites. Electron paramagnetic resonance (EPR) spin-trapping results showed that the highly reactive hydroxyl radical (·OH) was involved in the catalytic oxidation process. The spacer length had a direct effect on the catalytic efficiency of CoPc@s-BC—the decoloration rate for CoPc@TP-BC was as high as 41 µmol·min⁻¹·g⁻¹, which was more than 50% higher than that without spacer.

Keywords: bacterial cellulose; phthalocyanine; nanocomposite; spacer length; decoloration

1. Introduction

Nanocellulose has spawned increasing interest from broad fields, because this naturally occurring nanomaterial combines the advantages of cellulose, such as being hydrophilic, environmentally benign and having an easily tunable surface, with prominent features of nanosized materials, such as having a very high surface area to volume ratio and considerable modification possibility [1]. The preparation of nanocellulose ranges from “top-down” processing by isolation of natural cellulose to “bottom-up” processing by culture medium with certain bacteria [2,3]. The nanocellulose produced directly from bacteria is well known as bacterial cellulose (BC). Compared with “top-down” produced nanocellulose, BC is highly pure (hemicellulose- and lignin-free) and possesses unique and sophisticated three-dimensional, porous network structures which have been widely used in many fields. Potential applications of BC include mechanically reinforcement nanofillers [4,5], medical products [6], wound treatments [7], bio-inspired nanomaterials [8], and reaction templates [9]. In particular, its well-defined 3D network structures, high surface-area-to-volume-ratio, high accessibility, remarkable functionality, excellent mechanical properties and sustainability suggest
that BC is highly suitable as a template or substrate for the impregnation of a range of functional guest molecules, such as metal oxides, metal nanoparticles, mineral nanomaterials and carbonaceous nanomaterials [10,11].

Metal phthalocyanine complexes (MPcs), a class of versatile functional molecules [12], are promising catalysts for many processes because of their structural relations to naturally-occurring metal porphyrin complexes [13–16]. In practical applications, the immobilization of MPcs with solid substrates [17–22] is essential for convenient separation of catalysts from reaction media, long-term and continuous operation, overcoming the formation of inactive aggregates and preserving their activity. By carefully selecting appropriate substrate materials with specific microenvironment suitable for MPc reactions, their catalytic activity can be markedly enhanced. BC is undoubtedly a promising candidate for MPc immobilization, owing to the advantageous features mentioned above. Our group has been working on MPc immobilization using BC as a substrate [23–25], and we have comprehensively investigated the immobilization of MPcs onto BC.

Despite the enormous strides made in this research field, immobilized MPcs catalysts often suffer from various disadvantages of heterogeneous reaction conditions when compared with their homogeneous counterparts [26]. Direct immobilization of MPcs onto substrate surfaces will inevitably lead to the mass transfer limitation of target molecules to the heterogeneous catalysts [27–29]. Steric hindrance between the substrates and the catalysts also disfavors the catalytic reaction [30,31]. To overcome these drawbacks, an appropriate long and flexible spacer arm can be employed to keep the MPc molecules at a reasonable distance from the substrate surface. The presence of the spacer may mitigate the unfavorable effect of diffusion limitation and steric hindrance, enhance the accessibility and mobility of the MPc catalysts and increase the reaction homogeneity, thus increasing the catalytic activity. Spacers with variable lengths have been widely employed by researchers to enhance different kinds of catalysts. An increase in spacer length can efficiently enhance the catalytic activities of catalysts, such as protoporphyrin photosensitizers [32], zirconium complexes [33], and enzymes [34,35]. A few examples of spacers that can influence the electron transfer activity of MPcs have been reported [36–38]. However, little has been reported specifically concerning the effect of spacer length on the catalytic activity of MPcs. An investigation of how the spacer length influence the MPcs’ catalytic efficiency is, therefore, of timely importance.

The present study aimed to combine the advantages of BC and the flexible spacer for the preparation of a series of spacer-incorporated, tetra-amino cobalt (II) phthalocyanine (CoPc)-immobilized bacterial cellulose (BC) functional nanocomposites (CoPc@s-BC). A range of flexible spacers with different lengths, namely diethylenetriamine (DT), triethylenetetramine (TT), tetraethylenepentamine (TP) and pentaethylenehexamine (PH) (Figure 1), were attached onto BC for the subsequent covalent immobilization of the CoPc catalyst. The resulting nanocomposites were employed for the decoloration of dye wastewater; the synergistic improvement of both the adsorption capacity and catalytic activity of CoPc was expected. The mechanism of the catalytic oxidation decoloration was investigated using the electron paramagnetic resonance (EPR) spin-trapping technique. The effect of the spacer length on the decoloration efficiency was carefully studied. This research illustrates the key roles of the spacer and the spacer length in the enhancement of catalytic activity of MPc and offers a new perspective into the design of a highly efficient catalytic system for the remediation of dye wastewater.
was determined with the above-mentioned ninhydrin method. (4) CoPc@s-BC was harvested by submerging the glutaraldehyde-activated BC in a 2×10−3 mol/L CoPc solution and reacted for 2 h at 25 °C. The product was washed 3 times with dimethylformamide, 3 times with ultrapure water and then dried at 50 °C for 12 h. The content of immobilized CoPc was calculated according to the cobalt content on CoPc@s-BC, measured by atomic absorption spectrometry (Thermo solar M6, Thermo Fisher). The spin-trapping reagent, 5,5-dimethyl-1-pyrroline-N-oxide (DMPO), was purchased from Sigma Chemical Co. (Saint Louis, MO, USA). Ninhydrin reagent, DT, TT, TP, PH and all other chemicals were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China) and used without further purification.

2. Materials and Methods

2.1. Materials and Reagents

Acetobacter xylinum was produced by cultivating Acetobacter xylinum bacteria in a liquid culture medium containing 8.0 w/v % D-glucose, 1.0 w/v % yeast extract and 1.0 v/v % ethanol, as reported in the literature [39]. CoPc was synthesized from 4-nitrophthalic acid, urea and cobalt chloride hexahydrate, according to a method described previously [40,41]. Reactive red X-3B (C.I. Reactive Red 2, C19H19Cl2N2Na2O7S2, M.W.: 615.33) was purchased from Shanghai Chemical Reagent Factory. The spin-trapping reagent, 5,5-dimethyl-1-pyrroline-N-oxide (DMPO), was purchased from Sigma Chemical Co. (Saint Louis, MO, USA). Ninhydrin reagent, DT, TT, TP, PH and all other chemicals were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China) and used without further purification.

2.2. Preparation and Characterization of CoPc@s-BC

To prepare CoPc@s-BC, the following steps were carried out: (1) 20 mg of pure BC was incubated with a 30 mmol/L NaIO4 solution and reacted for 8 h. The resulting oxidized BC was thoroughly washed with ultrapure water and dried at 50 °C for 12 h. The content of aldehyde groups on oxidized BC was determined according to the literatures with minor modifications [42,43]. (2) The dried, oxidized BC was submerged into a spacer solution (DT or TT or TP or PH) and shaken on a temperature-controlled shaker. The content of the attached spacer was indirectly determined by measuring the number of amino groups on the BC substrate using a ninhydrin-based monitoring system, as described in the literature [30]. Ninhydrin reagent can react with primary amines to form a colored complex which is soluble in C2H5OH/H2O mixed solvent and highly conjugated, with a strong absorption at ca. 570 nm. Two hundred milliliters of ninhydrin reagent and 100 μL of H2O were added to 1 mg of spacer-attached BC and heated in a 100 °C water bath for 1 h. Five milliliters of C2H5OH/H2O (50:50, v/v) mixed solvent was added, and the mixture was analyzed on a UV-vis absorption spectrometer (UV-2450) at the wavelength of maximum absorbance: 570 nm. (3) The spacer-attached BC was submersed into 12 mL of glutaraldehyde solution and shaken at 25 °C for 2 h. The glutaraldehyde-activated BC was washed 3 times with ultrapure water and then dried at 50 °C for 12 h. The residual attached spacer content after glutaraldehyde activation was determined with the above-mentioned ninhydrin method. (4) CoPc@s-BC was harvested by submerging the glutaraldehyde-activated BC in a 2×10−2 mol/L CoPc solution and reacted for 2 h at 25 °C. The product was washed 3 times with dimethylformamide, 3 times with ultrapure water and then dried at 50 °C for 12 h. The content of immobilized CoPc was calculated according to the cobalt content on CoPc@s-BC, measured by atomic absorption spectrometry (Thermo solar M6, Thermo Fisher) with minor modifications [42,43].

Figure 1. Chemical structures of (a) diethylenetriamine (DT); (b) triethylenetetramine (TT); (c) tetraethylenepentamine (TP); and (d) pentaethylenhexamine (PH).
Fisher, Waltham, MA, USA). All of the modification and functionalization processes are schematically shown in Figure 2. The elemental compositions and chemical bonding of BC, oxidized BC, TP-attached BC, glutaraldehyde activated BC and CoPc@TP-BC were analyzed by X-ray photoelectron spectroscopy (XPS). XPS spectra were recorded on a Kratos Axis Ultra XPS system with Al (mono) Ka irradiation (hv = 1486.6 eV). The binding energy peaks of the XPS spectra were calibrated by placing the principal C 1s binding energy peak at 284.6 eV. 

![Figure 2: Synthesis route of spacer-incorporated, tetra-amino cobalt (II) phthalocyanine immobilized bacterial cellulose nanocomposites (CoPc@s-BC).](image)

2.3. Adsorption and Catalytic Oxidation Decoloration

The adsorption of reactive red X-3B was carried out in a glass flask sealed in a water bath at 50 °C. One milligram of CoPc@s-BC was added to 5 mL of reactive red X-3B dye wastewater (100 μmol/L, pH = 2). The catalytic oxidation was initiated by adding 8 mM H2O2 to the CoPc@s-BC containing the reactive red X-3B solution. At given time intervals, the samples were analyzed immediately on a UV-vis spectrophotometer (UV-2450) at the wavelength of maximum absorbance: 539 nm. The decoloration of reactive red X-3B was expressed as the change in the (C0 - C)/C0 value, where C0 is the initial concentration of the dye, and C is the residual concentration of the dye. The amount of adsorbed dye was calculated as follows:

$$\text{Adsorbed dye (μmol) = 100 μmol/L × 5 × 10^{-3} L × \frac{C_0 - C}{C_0}.}$$

(1)

The adsorption rate of CoPc@s-BC was derived from the slope of the adsorbed dye-adsorption time curves (within 90 min). The decoloration rate of CoPc@s-BC was calculated using Formula (2):

$$\text{Decoloration rate (μmol × min}^{-1} \times g}^{-1} = \frac{100 \text{μmol/L} \times 5 \times 10^{-3} \text{L} \times 90\%}{t \text{ min} \times 1 \times 10^{-3} \text{g} \times C_{\text{CoPc}} \times 630 \text{g/mol}},$$

(2)

where t is the time taken for decoloration of 90% of reactive red X-3B, and C_{CoPc} is the immobilized CoPc content of CoPc@s-BC. The EPR signal of radical spin-trapped by DMPO was detected with a Bruker-A300 X-band EPR spectrometer (Bruker, Karlsruhe, Germany).

3. Results and Discussion

3.1. Preparation and Characterization of CoPc@s-BC

CoPc@s-BC nanocomposites were prepared by covalent immobilization of CoPc onto the nanofibers after BC had been oxidized with NaIO4; this was spacer-attached with a series of flexible spacers and activated with glutaraldehyde. All the functionalization reactions were monitored by...
X-ray photoelectron spectrum (XPS), with the preparation of CoPc@TP-BC as a typical representative (Figure 3), and the chemical compositions of the samples during the series of reactions were calculated and are shown in Table 1. For the as-prepared pure BC, the two characteristic peaks at 284.6 eV and 530.6 eV were ascribed to the binding energies of C 1s and O 1s, respectively (Figure 3a). No significant changes were found after NaO4 oxidation (Figure 3b). Upon the TP attachment process, an additional peak at ca. 400 eV was detected, which corresponds to the characteristic peak of N 1s, implying the successful attachment of the TP spacer onto the oxidized BC (Figure 3c). The glutaraldehyde activation step did not obviously change the relative fractions of C, O and N elements (Figure 3d and Table 1). After the CoPc functionalization process, a marked increase (from 6.91% to 19%) of N 1s peak was found (Figure 3e and Table 1); Furthermore, two new characteristic peaks at 779.6 eV and 795.3 eV were also detected (Figure 3e, inset). These two peaks were assigned to the binding energies of Co 2p3/2 and Co 2p1/2, respectively. These results verify the success of all of the functionalization processes and the immobilization of CoPc on BC.

![XPS spectra](image)

**Figure 3.** XPS spectra of (a): bacterial cellulose (BC); (b): oxidized BC; (c): tetraethylenepentamine (TP)-attached BC; (d): glutaraldehyde-activated BC and (e): CoPc@TP-BC. The window included shows, in detail, the Co region of CoPc@TP-BC.

**Table 1.** Chemical compositions of BC during the functionalization processes.

|                          | C (mol %) | O (mol %) | N (mol %) | Co (mol %) |
|--------------------------|-----------|-----------|-----------|------------|
| BC                       | 63.13     | 36.87     | -         | -          |
| Oxidized BC              | 65.51     | 34.49     | -         | -          |
| TP-attached BC           | 63.55     | 29.61     | 6.84      | -          |
| Glutaraldehyde-activated BC | 64.53   | 28.56     | 6.91      | -          |
| CoPc@TP-BC               | 72.44     | 7.54      | 19        | 1.01       |

3.2. Optimization of CoPc Immobilization Conditions

For real applications, the optimum CoPc immobilization content on the BC substrate is desired to enhance its catalytic reaction efficiency without increasing the cost. The immobilized CoPc content directly depends on the amount of attached spacer; hence, the reaction condition for spacer attachment should be carefully monitored prior to CoPc immobilization.

The attachment levels of different spacers on the oxidized BC substrate as a function of reaction time is illustrated in Figure 4. The attached amount of all spacers increased almost directly proportionally with time, from 0 to 4 h, suggesting that the spacer attachment rate was initially constant. It is interesting that there appears to be a direct correlation between the attachment level and the spacer.
length, with longer spacers having lower amounts of spacer attached. The difference in attachment level increased with time. After reacting for 10 h, the attached amounts of TT, TP, and PH spacers were \(~2.2\) mmol/g, \(~2\) mmol/g and \(~1.7\) mmol/g, which were ca. 10%, 15% and 30% lower than that of the DT spacer (\(~2.4\) mmol/g), respectively. Further prolongation of the reaction time did not consistently increase the attached spacer content on oxidized BC. Considering both the attachment level and the attachment efficiency, the optimum attachment time was set to 10 h.

![Image](Appl. Sci. 2018, 8, x FOR PEER REVIEW 6 of 14)

**Figure 4.** Effect of the reaction time on the amount of attached spacer on the oxidized BC, \(T = 30\) °C (40 °C for PH), initial spacer concentration = 8% (v/v).

Our previous work illustrated that under optimum oxidation conditions, oxidized BC contains 14.13% (wt./wt.) of aldehyde groups (ca. 5 mmol/g, slightly lower than the theoretical maximum content of 18.1% (wt./wt.)) [25], while the amount of attached spacer was lower than 2.5 mmol/g; that is, less than half of the aldehyde groups were effectively utilized for the attachment of spacers. The relatively low-efficiency usage of aldehyde groups can be ascribed to two reasons. The first reason is the difficulty of producing heterogeneous reactions and the incompleteness characteristic of macromolecular reactions. The second reason may be attributed, at least partly, to the steric hindrance effect; that is, when the amount of attached spacer increased to a certain level, the already fixed spacers prevented further progress of the attachment reaction. It is reasonable to assume that the most obvious steric hindrance effect arises from PH, i.e., the longest spacer. Given the above factors, the attachment levels for all of the spacers were much smaller than the theoretical value, and the PH spacer had the lowest attached content.

The spacer-attached BC substrates were further activated with glutaraldehyde, a classic bifunctional chemical crosslinker, for the immobilization of the CoPc catalyst. The immobilized CoPc contents were affected by many factors, such as the contents of the attached spacers and the activation levels. Figure 5a demonstrates the influence of initial glutaraldehyde concentration on the immobilized CoPc content for DT-attached BC. The immobilized CoPc content increased in association with the increase of glutaraldehyde concentration. It is reasonable to assume that with sufficient activation reagents, more binding sites were provided for CoPc immobilization. Meanwhile, the amount of DT spacer decreased drastically, which statistically represents the successfully activation of glutaraldehyde for CoPc immobilization. Two percent glutaraldehyde was required for the maximum immobilization of CoPc; accordingly, the content of immobilized CoPc was as high as ca. 550 μmol/g, and the residual DT spacer was 0.38 mmol/g. A further increase in the activation reagent did not lead to the disappearance of the DT spacer (even when the glutaraldehyde concentration reached 5%), which may be ascribed to the incompleteness of the macromolecular reaction. Similar results were
found for the immobilization of CoPc onto TT-attached BC (Figure 5b), TP-attached BC (Figure 5c) and PH-attached BC (Figure 5d).

Figure 5. Effect of the glutaraldehyde concentration on the immobilized CoPc and residual spacer content for (a) DT-attached BC; (b) TT-attached BC; (c) TP-attached BC; and (d) PH-attached BC, $T = 25 \, ^\circ C$, reaction time = 2 h.

3.3. Adsorption and Catalytic Oxidation Performance of CoPc@s-BC

The prepared series of CoPc@s-BC were aimed at being functional nanocomposites for the decoloration of dye wastewater. The total decoloration includes the adsorption of dye molecules and the subsequent catalytic oxidation process [40]. The performances of the prepared CoPc@s-BC nanocomposites were firstly evaluated based on their adsorption behavior towards reactive red X-3B dye wastewater (Figure 6). For pure BC, only 0.05 μmol of the dye molecules were adsorbed after a dynamic equilibrium, reached in 240 min. CoPc@BC had a much higher adsorption capacity than pure BC—0.2 μmol of dye was adsorbed by CoPc@BC under the same experimental conditions. The adsorption capacities of all the spacer-attached nanocomposites were higher when compared with CoPc@BC. After dynamic equilibrium was reached, the amounts of the adsorbed dye by CoPc@DT-BC, CoPc@TT-BC, CoPc@TP-BC and CoPc@PH-BC were 37%, 54%, 67% and 17% higher than that of CoPc@BC, respectively. As illustrated in Figure 5, the contents of immobilized CoPc for CoPc@DT-BC, CoPc@TT-BC, CoPc@TP-BC and CoPc@PH-BC were 27%, 21%, 15% and 7% higher than that of CoPc@BC, respectively. By comparing these statistics, it is easy to deduce that the enhancement of adsorption capacity was mainly caused by the incorporation of spacers. The incorporation of spacers caused CoPc to immobilize onto BC in a more dispersed way, which effectively increased the number of contact opportunities between CoPc and the dye molecules; furthermore, the diffusion limitation of dye molecules to CoPc was effectively reduced. These effects were correlated with the spacer length. CoPc@TP-BC has a longer spacer than CoPc@DT-BC and CoPc@TT-BC, and its adsorption capacity was
much higher. However, it is possible that when the spacer was too long, a CoPc might have attached onto BC through multiple-bonding, and the peripheral amino groups on CoPc (which was necessary for proton acceptance) [40] may have been excessively consumed; thus the adsorption capacity may have been reduced to some extent. The CoPc@PH-BC has the longest incorporated spacer, while its adsorption capacity was relatively lower than others.

![Figure 6](image)

**Figure 6.** Adsorption of reactive red X-3B wastewater (initial concentration 100 μM, pH = 2, T = 50 °C) with CoPc@s-BC (1 mg).

To determine the adsorption rate of CoPc@s-BC, the adsorption curves in Figure 6 were regressed using a linear function (within 90 min); the parameters, including the slope and correlation ratio, are presented in Table 2. The amount of adsorbed dye as a function of adsorption time showed a linear trend; all correlation ratios (r) for the CoPc@s-BC nanocomposites were >0.99. The incorporation of spacers greatly facilitated the contact between the CoPc and the dye molecules—all of the CoPc@s-BC had higher adsorption rates than CoPc@BC. The adsorption rate of CoPc@TP-BC reached 0.385 μmol/min, which was more than two times higher than that of CoPc@BC (0.172 μmol/min).

| Sample           | Slope     | Correlation Ratio |
|------------------|-----------|-------------------|
| BC               | 0.0563    | 0.9668            |
| CoPc@BC          | 0.1723    | 0.9946            |
| CoPc@DT-BC       | 0.2453    | 0.9921            |
| CoPc@TT-BC       | 0.3466    | 0.9955            |
| CoPc@TP-BC       | 0.3852    | 0.9942            |
| CoPc@PH-BC       | 0.2240    | 0.9985            |

Table 2. Slope and correlation ratios regressed from the adsorbed dye-adsorption time curves.

One of the main concerns for this study was to evaluate the effects of the spacers, especially the spacer length, on the catalytic activity of the CoPc catalyst. All series of CoPc@s-BC were employed for the catalytic oxidation of reactive red X-3B dye wastewater, with H₂O₂ as an oxidant. The investigation of the influence of spacers on the catalytic oxidation rate revealed similar results to that of adsorption behavior. A control experiment was firstly conducted. H₂O₂ alone showed almost no decoloration activity—it was difficult to decompose reactive red X-3B with H₂O₂ without CoPc@s-BC (Figure 7). All of the prepared CoPc@s-BC nanocomposites are able to catalytic decolorize dye wastewater, and the decoloration rate was correlation with the spacer length (Figure 7, inset). The typical optical image changes in reactive red X-3B solution during the decoloration process are shown in Figure 8.
For CoPc@BC, the decoloration rate was ca. 27 $\mu$mol·min$^{-1}$·g$^{-1}$. With the incorporation of the spacer, enhanced catalytic activity of CoPc catalyst was observed. Under the same reaction conditions, the decoloration rate of CoPc@DT-BC was ca. 8% higher than that of CoPc@BC. Much higher catalytic activity of CoPc was obtained with the incorporation of a longer spacer length. The decoloration rates of CoPc@TT-BC and CoPc@TP-BC were ca. 33 $\mu$mol·min$^{-1}$·g$^{-1}$ and 41 $\mu$mol·min$^{-1}$·g$^{-1}$, which were more than 20% and 50% higher than that of CoPc@BC, respectively. This result is in good accordance with our principle idea—that the incorporation of a flexible spacer is able to keep the CoPc catalyst at a preferred distance from the solid substrate, which, to some extent, mitigates the adverse effects of diffusion limitation and steric hindrance, increases the reaction homogeneity and enhances the accessibility and mobility of the CoPc catalyst. These effects are more likely to occur with longer spacer lengths. However, when the spacer is too long, it is possible that the CoPc molecules covalently immobilize through multiple-bonding, which restricts the free movement of the CoPc and thus, decreases its catalytic activity to some extent. The decoloration rate for CoPc@PH-BC was ca. 33 $\mu$mol·min$^{-1}$·g$^{-1}$, which was higher than CoPc@BC but lower than CoPc@TP-BC.

![Figure 7](image_url)  
**Figure 7.** Catalytic oxidation of reactive red X-3B (initial concentration 100 $\mu$M, pH = 2, T = 50 °C) with CoPc@s-BC (1 mg) and H$_2$O$_2$ (8 mM). Inset: effect of the spacer length on the decoloration rate of reactive red X-3B.

![Figure 8](image_url)  
**Figure 8.** Typical optical image changes of reactive red X-3B (initial concentration 100 $\mu$M, pH = 2, T = 50 °C) with CoPc@s-BC (1 mg) and H$_2$O$_2$ (8 mM).

To sum up, the most preferable spacer length to achieve the maximum decoloration efficiency of CoPc@s-BC was derived from the TP spacer. Under optimum immobilization conditions, 2 mmol/g of attached TP together with 495 $\mu$mol/g of immobilized CoPc were obtained, and the decoloration
rate for the resulting CoPc@TP-BC reached 41 \mu mol \cdot min^{-1} \cdot g^{-1}. These results are comparable with related literature. Firstly, flexible spacer-attached BCs were employed to support the CoPc catalyst, which permitted a much higher amount of immobilized CoPc than other materials [44,45]. Secondly, the incorporation of the spacers increased both the adsorption rate and the adsorption capacity of the nanocomposites [46]. Moreover, the incorporation of the spacers (especially for TP) effectively increased the accessibility and reaction homogeneity of the CoPc catalyst, and thus, its catalytic activity was greatly improved compared to that without the spacer [23].

The EPR spin-trapping technique is a powerful tool for the detection of short-lived, active species. Herein, the 5,5-dimethyl-1-pyrroline-N-oxide (DMPO)-trapped EPR spectra were employed to demonstrate the formation of radicals during the catalytic oxidation of reactive red X-3B with the CoPc@s-BC (CoPc@BC)/H$_2$O$_2$ reaction system (Figure 9). Without the existence of H$_2$O$_2$, no obvious EPR signal was detected. When both CoPc@BC and H$_2$O$_2$ were added, a strong, four-line spectrum with a peak intensity of 1:2:2:1 was easily detected, which is the typical characteristic spectrum of the DMPO-·OH adducts [47]. This result indicates that ·OH was generated during the reaction and was responsible for the catalytic oxidation of reactive red X-3B. Similar results were found for the CoPc@s-BC/H$_2$O$_2$ reaction system; the only differences were the peak intensities, which were proportional to the amount of the formed ·OH radical during the reaction. An obvious higher amount of ·OH was observed for the CoPc@TP-BC/H$_2$O$_2$ reaction system. Therefore, the CoPc@TP-BC had the highest catalytic activity for the decoloration of reactive red X-3B dye wastewater, which is in good accordance with the results illustrated in Figure 7.

![Figure 9](image_url)  
Figure 9. 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) spin-trapping electron paramagnetic resonance (EPR) spectra of reactive red X-3B dye wastewater (initial concentration 100 \mu M, pH = 2, T = 50 °C). The inset shows the magnification of the marked region.

Based on the above experimental results, a possible catalytic reaction mechanism is proposed in Figure 10. Firstly, the reactive red X-3B dye molecules were adsorbed onto the nanocomposites from solution. Thanks to the incorporation of flexible spacers, the contact between dye molecules and the CoPc pendants became easier; therefore, both the adsorption capacity and the adsorption rate were enhanced by the spacers. Secondly, the adsorbed dyes were catalytically decolorized in-situ by the ·OH radical. Due to the presence of spacers, the free movement and the reaction homogeneity of CoPc on CoPc@s-BC were improved; thus, more ·OH is produced during the oxidation of dye molecules.
These two processes cooperate in a synergistic manner and effective enhancement of the catalytic efficiency of CoPc can be reasonably expected. The decoloration was significantly associated with the spacer length of CoPc@s-BC. The most preferable spacer length arose from TP; thus, CoPc@TP-BC has the highest catalytic efficiency.

4. Conclusions

BCs attached with varying lengths of flexible spacers were prepared for the immobilization of CoPc and the fabrication of novel, spacer-incorporated, CoPc-immobilized CoPc@s-BC nanocomposites. These nanocomposites are promising materials for the decoloration of reactive dye wastewater. The incorporation of spacers effectively increased the decoloration efficiency of CoPc@s-BC. The most preferable spacer length for optimizing both the adsorption capacity and the catalytic oxidation efficiency was derived from the TP spacer. With the incorporation of TP, the adsorption capacity of CoPc@TP-BC was 67\% higher than that of CoPc@BC, and its adsorption rate increased by more than two times. The CoPc@TP-BC was able to efficiently decolorize reactive dye wastewater with H2O2 as an oxidant, and the decoloration rate was as high as 41 \( \mu \text{mol min}^{-1} \cdot \text{g}^{-1} \), ca. 50\% higher than that of CoPc@BC. EPR spin-trapping experiments revealed that highly reactive \( \cdot \text{OH} \) is responsible for the catalytic oxidation reaction. The incorporation of spacers boosts the formation of \( \cdot \text{OH} \) and thus increases the catalytic activity of CoPc@s-BC.

Author Contributions: S.C. conceived the study, designed the experiments, analyzed the data and wrote the paper. Q.T. performed the experiments. W.X. designed the experiments and analyzed the data.

Funding: This work was supported by the Zhejiang Provincial Natural Science Foundation of China (Grant no. LQ15E030005), Innovation Training Plan of Zhejiang Province for University Students (Xinmiao Talent Programme 2017R423028) and Research Programme of Qianjiang College (2018QJJL04).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Nechyporchuk, O.; Belgacem, M.N.; Bras, J. Production of cellulose nanofibrils: A review of recent advances. Ind. Crops Prod. 2016, 93, 2–25. [CrossRef]
2. Klemm, D.; Kramer, F.; Moritz, S.; Lindström, T.; Ankerfors, M.; Gray, D.; Dorris, A. Nanocelluloses: A new family of nature-based materials. Angew. Chem. Int. Ed. 2011, 50, 5438–5466. [CrossRef] [PubMed]
3. Eichhorn, S.J.; Dufresne, A.; Aranguren, M.; Marcovich, N.E.; Capadona, J.R.; Rowan, S.J.; Weder, C.; Thielemans, W.; Roman, M.; Renneckar, S.; et al. Review: Current international research into cellulose nanofibres and nanocomposites. J. Mater. Sci. 2010, 45, 1–33. [CrossRef]
4. Wang, J.Y.; Jia, H.B.; Zhang, J.J.; Ding, L.F.; Huang, Y.; Sun, D.P.; Gong, X.D. Bacterial cellulose whisker as a reinforcing filler for carboxylated acrylonitrile-butadiene rubber. J. Mater. Sci. 2014, 49, 6093–6101. [CrossRef]

5. Wang, B.; Yang, D.; Zhang, H.R.; Huang, C.; Xiong, L.; Luo, J.; Chen, X.D. Preparation of esterified bacterial cellulose for improved mechanical properties and the microstructure of isotactic polypropylene/bacterial cellulose composites. Polymers 2016, 8, 129. [CrossRef]

6. Lin, N.; Dufresne, A. Nanocellulose in biomedicine: Current status and future prospect. Eur. Polym. J. 2014, 59, 302–325. [CrossRef]

7. Sulaeva, I.; Henniges, U.; Rosenau, T.; Potthast, A. Bacterial cellulose as a material for wound treatment: Properties and modifications. A review. Biotechnol. Adv. 2015, 33, 1547–1571. [CrossRef] [PubMed]

8. Capadona, J.R.; Shamgunanathan, K.; Tyler, D.J.; Rowan, S.J.; Weder, C. Stimuli-responsive polymer nanocomposites inspired by the sea cucumber dermis. Science 2008, 319, 1370–1374. [CrossRef] [PubMed]

9. Abeer, M.M.; Amin, M.C.I.M.; Lazim, A.M.; Pandey, M.; Martin, C. Synthesis of a novel acylated abietic acid-g-bacterial cellulose hydrogel by gamma irradiation. Carbohydr. Polym. 2014, 110, 505–512. [CrossRef] [PubMed]

10. Foresti, M.L.; Vazquez, A.; Boury, B. Applications of bacterial cellulose as precursor of carbon and composites with metal oxide, metal sulfide and metal nanoparticles: A review of recent advances. Carbohydr. Polym. 2017, 157, 447–467. [CrossRef] [PubMed]

11. Wei, H.; Rodriguez, K.; Renneckar, S.; Vikesland, P.J. Environmental science and engineering applications of nanocellulose-based nanocomposites. Environ. Sci.-Nano 2014, 1, 302–316. [CrossRef]

12. De la Torre, G.; Claessens, C.G.; Torres, T. Phthalocyanines: Old dyes, new materials. Putting color in nanotechnology. Chem. Commun. 2007, 2000–2015. [CrossRef]

13. Sorokin, A.B. Phthalocyanine metal complexes in catalysis. Chem. Rev. 2013, 113, 8152–8191. [CrossRef] [PubMed]

14. Sorokin, A.; Seris, J.L.; Meunier, B. Efficient oxidative dechlorination and aromatic ring-cleavage of chlorinated phenols catalyzed by iron sulphotphalocyanine. Science 1995, 268, 1163–1166. [CrossRef] [PubMed]

15. Sorokin, A.B.; Kudrik, E.V. Phthalocyanine metal complexes: Versatile catalysts for selective oxidation and bleaching. Catal. Today 2011, 159, 37–46. [CrossRef]

16. Chen, X.; Lu, W.Y.; Xu, T.F.; Li, N.; Qin, D.D.; Zhu, Z.X.; Wang, G.Q.; Chen, W.X. A bio-inspired strategy to enhance the photocatalytic performance of g-c3n4 under solar irradiation by axial coordination with hemin. Appl. Catal. B Environ. 2017, 201, 518–526. [CrossRef]

17. Zhu, Z.X.; Chen, Y.; Gu, Y.; Wu, F.; Lu, W.Y.; Xu, T.F.; Chen, W.X. Catalytic degradation of recalcitrant pollutants by fenton-like process using polyacrylonitrile-supported iron (ii) phthalocyanine nanofibers: Intermediates and pathway. Water Res. 2016, 93, 296–305. [CrossRef] [PubMed]

18. Da Silva, T.H.; de Souza, T.F.M.; Ribeiro, A.O.; Calefi, P.S.; Ciuffi, K.J.; Nasser, E.J.; Molina, E.F.; Hamer, P.; de Faria, E.H. New strategies for synthesis and immobilization of methalophtalocyanines onto kaolinite: Preparation, characterization and chemical stability evaluation. Dyes Pigments 2016, 134, 41–50. [CrossRef]

19. Yang, W.X.; Zhang, R.L.; Luo, K.; Zhang, W.P.; Zhao, J.S. Electrocatalytic performances of multi-walled carbon nanotubes chemically modified by metal phthalocyanines in li/socl2 batteries. RSC Adv. 2016, 6, 75632–75639. [CrossRef]

20. De Wael, K.; Adriaens, A. Phthalocyanines and porphyrins linked to gold adatoms and their catalytic property towards hydroxide oxidation. Electrochim. Acta 2008, 53, 2355–2361. [CrossRef]

21. Balkus, K.J.; Eissa, M.; Levado, R. Oxidation of alkanes catalyzed by zeolite-encapsulated perfluorinated ruthenium phthalocyanines. J. Am. Chem. Soc. 1995, 117, 10753–10754. [CrossRef]

22. Han, Z.B.; Han, X.; Zhao, X.M.; Yu, J.T.; Xu, H. Iron phthalocyanine supported on amidoximated pan fiber as effective catalyst for controllable hydrogen peroxide activation in oxidizing organic dyes. J. Hazard. Mater. 2016, 320, 27–35. [CrossRef] [PubMed]

23. Chen, S.; Huang, Y. Bacterial cellulose nanofibers decorated with phthalocyanine: Preparation, characterization and dye removal performance. Mater. Lett. 2015, 142, 235–237. [CrossRef]

24. Chen, S.; Huang, Y.; Huang, J. Novel preparation of multiwalled carbon nanotubes/bacterial cellulose nanocomposite for phthalocyanine immobilization. Funct. Mater. Lett. 2017, 10, 1750038. [CrossRef]
25. Chen, S.; Teng, Q. Quantitative immobilization of phthalocyanine onto bacterial cellulose for construction of a high-performance catalytic membrane reactor. *Materials* 2017, 10, 846. [CrossRef] [PubMed]

26. Dyer, P.W.; Handa, S.; Reeve, T.B.; Suhard, S. The synthesis and catalytic application of spacer-modified diol-functionalised merffield resins. *Tetrahedron Lett.* 2005, 46, 4753–4756. [CrossRef]

27. Chen, S.; Huang, X.; Xu, Z. Effect of a spacer on phthalocyanine functionalized cellulose nanofiber mats for decolorizing reactive dye wastewater. *Cellulose* 2012, 19, 1351–1359. [CrossRef]

28. Wang, F.; Gu, Z.G.; Cui, Z.G.; Liu, L.M. Comparison of covalent immobilization of amylase on polystyrene pellets with pentaethylenexamine and pentaethylene glycol spacers. *Bioresour. Technol.* 2011, 102, 9374–9379. [CrossRef] [PubMed]

29. Alptekin, O.; Tutel, S.S.; Yildirim, D.; Alagöz, D. Covalent immobilization of catalase onto spacer-arm attached modified florisil: Characterization and application to batch and plug-flow type reactor systems. *Enzym. Microb. Technol.* 2011, 49, 547–554. [CrossRef] [PubMed]

30. Ozylmaz, G. The effect of spacer arm on hydrolytic and synthetic activity of candida rugosa lipase immobilized on silica gel. *J. Mol. Catal. B Enzym.* 2009, 56, 231–236. [CrossRef]

31. Fernandez-Lorente, G.; Palomo, J.M.; Cabrera, Z.; Guisan, J.M.; Fernandez-Lafuente, R. Specificity enhancement towards hydrophobic substrates by immobilization of lipases by interfacial activation on hydrophobic supports. *Enzym. Microb. Technol.* 2007, 41, 565–569. [CrossRef]

32. Zhu, J.; Sun, G. Preparation and photo-oxidative functions of poly(ethylene-co-methacrylic acid) (pe-co-maa) nanofibrous membrane supported porphyrins. *J. Mater. Chem.* 2012, 22, 10581–10588. [CrossRef]

33. Cho, W.S.; Kim, S.H.; Kim, D.J.; Mun, S.D.; Kim, R.; Go, M.J.; Park, M.H.; Kim, M.; Lee, J.; Kim, Y. Zirconium complexes with pendant arylxy groups attached to the metallocene moiety by ethyl or hexyl spacers. *Polyhedron* 2014, 67, 205–212. [CrossRef]

34. Stavrakov, G.; Philipova, I.; Zheleva, D.; Atanasova, M.; Konstantinov, S.; Doytchinova, I. Docking-based design of galantamine derivatives with dual-site binding to acetylcholinesterase. *Mol. Inform.* 2016, 35, 278–285. [CrossRef]

35. Verma, S.K.; Ghritlahre, B.K.; Ghosh, K.K.; Verma, R.; Verma, S.; Zhao, X.J. Influence of amine-based cationic gemini surfactants on catalytic activity of -chymotrypsin. *Int. J. Chem. Kinet.* 2016, 48, 779–784. [CrossRef]

36. Jimenez, A.J.; Marcos, M.L.; Hausmann, A.; Rodriguez-Morgade, M.S.; Guldi, D.M.; Torres, T. Assembling phthalocyanine dimers through a platinum(ii) acetylide linker. *Chem. Eur. J.* 2011, 17, 14139–14146. [CrossRef] [PubMed]

37. Lederer, M.; Ince, M.; Martinez-Diaz, M.V.; Torres, T.; Guldi, D.M. Photoinduced electron transfer in a zinc phthalocyanine-fullerene conjugate connected by a long flexible spacer. *Chemplischem* 2016, 81, 941–946. [CrossRef]

38. He, D.D.; Peng, Y.R.; Yang, H.Q.; Ma, D.D.; Wang, Y.H.; Chen, K.Z.; Chen, P.P.; Shi, J.F. Single-wall carbon nanotubes covalently linked with zinc (ii) phthalocyanine bearing poly (aryl benzyl ether) dendritic substituents: Synthesis, characterization and photoinduced electron transfer. *Dyes Pigments* 2013, 99, 395–401. [CrossRef]

39. Yang, J.; Sun, D.; Li, J.; Yang, X.; Yu, J.; Hao, Q.; Liu, W.; Liu, J.; Zou, Z.; Gu, J. In situ deposition of platinum nanoparticles on bacterial cellulose membranes and evaluation of pem fuel cell performance. *Electrochem. Acta* 2009, 54, 6300–6305. [CrossRef]

40. Chen, S.; Huang, X.; Xu, Z. Functionalization of cellulose nanofiber mats with phthalocyanine for decoloration of reactive dye wastewater. *Cellulose* 2011, 18, 1295–1303. [CrossRef]

41. Achar, B.; Fohlen, G.; Parker, J.; Keshavayya, J. Synthesis and structural studies of metal (ii) 4, 9, 16, 23-phthalocyanine tetraamines. *Polyhedron* 1987, 6, 1463–1467. [CrossRef]

42. Liu, H.Q.; Hsieh, Y.L. Ultrafine fibrous cellulose membranes from electrospinning of cellulose acetate. *J. Polym. Sci. Part B Polym. Phys.* 2002, 40, 2119–2129. [CrossRef]

43. Huang, X.J.; Chen, P.C.; Huang, F.; Ou, Y.; Chen, M.R.; Xu, Z.K. Immobilization of candida rugosa lipase on electrospun cellulose nanofiber membrane. *J. Mol. Catal. B Enzym.* 2011, 70, 95–100. [CrossRef]

44. Chen, W.X.; Lu, W.Y.; Yao, Y.Y.; Xu, M.H. Highly efficient decomposition of organic dyes by aqueous-fiber phase transfer and in situ catalytic oxidation, using fiber-supported cobalt phthalocyanine. *Environ. Sci. Technol.* 2007, 41, 6240–6245. [CrossRef] [PubMed]

45. Matama, T.; Araujo, R.; Gubitz, G.M.; Casal, M.; Cavaco-Paulo, A. Functionalization of cellulose acetate fibers with engineered cutinases. *Biotechnol. Prog.* 2010, 26, 636–643. [CrossRef] [PubMed]
46. Gao, M.P.; Lu, W.Y.; Li, N.; Chen, W.X. Enhanced removal of acid red 1 with large amounts of dyeing auxiliaries: The pivotal role of cellulose support. *Cellulose* **2014**, *21*, 2073–2087. [CrossRef]

47. Yamazaki, I.; Piette, L.H. EPR spin-trapping study on the oxidizing species formed in the reaction of the ferrous ion with hydrogen-peroxide. *J. Am. Chem. Soc.* **1991**, *113*, 7588–7593. [CrossRef]