Efficient Biodegradation of Azo Dyes Catalyzed by the Anthraquinone-2-sulfonate and Reduced Graphene Oxide Nanocomposite

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ABSTRACT: An anthraquinone-2-sulfonate and reduced graphene oxide nanocomposite (AQS@rGO) was prepared and the improvement on the biotic reduction of a pollutant, i.e., azo dye, was demonstrated. Electron paramagnetic resonance signal of the semi-quinone radical in the well-dispersed solid AQS@rGO solution was detected. Although the as-prepared AQS@rGO has a negligible adsorption capacity toward methyl orange (MO) dye, the decolorization efficiencies in both flask experiments and sequencing operation reactors in the presence of AQS@rGO were increased by more than 1.5 times as compared to that with graphene oxide, and an efficient and continuable catalytic effect on the decolorization of azo dyes in seven operation periods was maintained. The catalytic effect on reduction was caused by the formation of a space-charge layer, which facilitates the efficient electron transfer from the conductive rGO sheets to the C=O of the AQS molecule. The results suggested that the AQS@rGO may act as an efficient insoluble redox mediator, which is important for the pollution control by accelerating the extracellular electron transfer.

1. INTRODUCTION

Quinoid redox mediators (QRM) were reported extensively to accelerate the reductions of pollutants, which was due to that the quinoid structure may accept electrons during the anaerobic respiration of quinone respiratory bacteria (QRB), and the reduced quinone, i.e., hydroquinone would then chemically react with pollutants with a very fast rate. As the high cost and easy loss make the continuous addition of soluble redox mediators (RMs) really expensive, researchers were moving their attention to the immobilization methods of the QRM. For example, Cervantes et al. reported that three quinones could be linked with anion exchange resins by the sulfonated group; Guo, et al. stated that the anthraquinone could be entrapped in calcium alginate, polyvinyl alcohol, and agar. However, the quinoids were easily exchanged under high concentrations of anions, and the mechanical strength of the carrier was weak, which limited their wide applications.

Recently, insoluble RMs were of natural origin, chemically stable, and environmentally benign, e.g., natural materials, carbonaceous materials, and artificial polymers contain quinoid structure, which were reported to accelerate the extracellular electron transfer during the anoxic process resulting in a rapid reduction of contaminants. However, the low efficiency of mass transfer between the surface of the reported polymers and microorganisms restricted the whole reduction rate: (1) the reduction of repeated reduction of the quinoid structure were usually considered as a speed-limiting step during the degradation process, and (2) the direct contact model is the main electron transfer way for the reduction of the quinoid structure, whether by pili or cell membrane. Better dispersibility and electroconductivity of the materials is therefore needed to be explored to further
improve the transfer rates of the reducing equivalents from the solid surface to the soluble contaminants.

Graphene oxide (GO) may be a good precursor for the purpose due to the characteristics of abundant functional groups, nanoscale size, and large specific surface area. Preparation and modification of the graphene-based nanocomposites showed great potential in pollution control technologies, especially in adsorption. On the other hand, anthraquinone-2-sulfonate (AQS) was known to enhance the efficiency of extracellular electron transfer in many previous studies due to the active redox properties of the quinoid structure. Taking inspiration from this fact, a GO and AQS nanocomposite was prepared by hydrothermal synthesis through noncovalent $\pi-\pi$ stacking to activate and stabilize the RM groups grafted on GO. Although the formation of the GO and AQS complexes has been widely studied and the insoluble AQS@rGO nanocomposite has already been reported to serve as high-performance supercapacitors or as efficient sorbent for synthetic dyes, its function as a solid RM for the biotic reduction has not been investigated so far.

Therefore, an AQS@rGO nanocomposite was fabricated and evaluated as a solid RM to accelerate the reductions of pollutants in this study. On the other hand, azo dyes were widely used and discharged in many industrial wastewaters, causing environmental deterioration and threat to human health. The chromophore of azo dye, i.e., the $-N=N-$ bond, could accept electron under an anaerobic environment and then decolorize. Therefore, azo dye was usually chosen as a model pollutant for evaluation of the performance of RM. The result of this study provides a mechanism research on biotic reduction of azo dye with the as-prepared nanomaterials, which is of great environmental significance on the application feasibility of the as-prepared AQS@rGO.

2. RESULTS AND DISCUSSION

2.1. Characterization. The GO was first prepared from natural graphite powder via the well-known Hummers methods. As shown in Figure 1a, the characteristic peaks of GO at 1724, 1630, and 1053 cm$^{-1}$ were the C=O in the carboxyl group, C=O stretching in $-CONH$ bands, and C−O−C in the epoxide group, respectively. In the spectrum of AQS@rGO, the peak at 1724 cm$^{-1}$ disappeared, and the intensities of peaks at 1630 cm$^{-1}$ (C=O) and 1053 cm$^{-1}$ (in-plane bending vibration of C−H) were smaller compared to that of GO. Meanwhile, some characteristic peaks of AQS were presented in the AQS@rGO complex, especially for the peaks at 1089 cm$^{-1}$ (C−O vibrations of the alkoxy group) and...
The obtained C 1s and O 1s spectra result further confirmed the attachment of AQS on the rGO matrix. The TEM images of the as-prepared AQS@rGO show a structure with unobvious aggregation (Figure 1d,e), which is different from that in AQS@rGO samples. The increased relative intensity of the C 1s peak and the decreased relative intensity of the O 1s peak in the spectrum of GO compared to those in the spectrum of AQS@rGO (Figure S1b). The results were in line with the reduction of GO by hydrothermal reduction. As shown in Figure S1a in the Supporting Information (SI), the dominating peak (C=O) at 284.8 eV (C−C) is in AQS@rGO (Figure S1b). The results were in line with the detailed O 1s spectrum (Figure S1c,d) that the abundance of the peak at 533.2 eV (C=O) in GO is significantly higher than that in AQS@rGO samples. The obtained C 1s and O 1s spectra further confirmed the FTIR results that three characteristic peaks of the carboxyl-containing groups were weakened during the preparation process (Figure 1a). The detailed S 2p spectra are shown in Figure S1e,f, which is dominated by the SO3− group at 168.0 and 169.0 eV.

The thermal gravimetric analysis (TGA) of the samples were determined by TGA measurement from room temperature to 700 °C in a nitrogen atmosphere (Figure 1c). Pure AQS shows obvious weight loss before 200 °C and between 460 and 500 °C, which is due to the removal of adsorbed moisture and the carbonization of AQS molecules, respectively. The slow weight loss after 500 °C is due to the gradual elimination of sulfur- and oxygen-containing functionalities of the AQS-derived carbon. The same weight decreases between 460 and 500 °C in the AQS@rGO sample clearly verified the attachment of AQS on the rGO matrix. The TEM images of the as-prepared AQS@rGO showed an irregular, thin, and wrinkled film structure with unobvious aggregation (Figure 1d,e), which is similar to some previous studies. Moreover, as shown in Figure 1f, the C 1s, O 1s, and S 1s elements were identified in the electron energy loss spectroscopy (EELS) mapping pictures of the as-prepared AQS@rGO. The EELS results suggested that the functional groups containing oxygen, carbon, and sulfur were uniformly distributed on the AQS@rGO surface. This might further confirm that the AQS was successfully grafted and uniformly covered on the rGO sheet.

### Table 1. Adsorption Isotherms and Kinetics of Azo Dyes by GO

| azo dyes | Langmuir model | Freundlich model |
|----------|----------------|------------------|
|          | qmax (mg g⁻¹)  | K_L (L/g) | R² | Kf (mg⁻¹ L⁻¹/n) | 1/n | R² |
| MO       | 128.205        | 0.897     | 0.932 | 48.240 | 0.392 | 0.991 |
| AB113    | 201.203        | 0.126     | 0.892 | 23.103 | 0.741 | 0.953 |
| RB5      | 28.898         | 0.561     | 0.943 | 11.824 | 0.284 | 0.967 |

"Langmuir isotherm model q = \( \frac{q_{\text{m}}K_{\text{L}}C}{1 + K_{\text{L}}C} \) where C is the equilibrium concentration of azo dyes (mg l⁻¹), q is the amount of azo dyes absorbed per gram of the as-prepared materials at equilibrium (mg g⁻¹), and qmax (mg g⁻¹) and K_L (L mg⁻¹) are the maximum adsorption capacity and Langmuir constant, respectively. Freundlich isotherm model q = \( K_{\text{F}}C_1^{1/n} \), where K_F and n are isotherm constants, which indicate the adsorption capacity (mg l⁻¹ L⁻¹/n g⁻¹) and the intensity of the adsorption, respectively. Pseudo first-order kinetics model ln(qe − qt) = ln qe − kt where k_1 and qe are the pseudo first-order rate constant (h⁻¹) and the amount of absorbed azo dyes (mg g⁻¹) at time t (h), respectively. Pseudo second-order kinetics model \( \frac{t}{q_{\text{e,cal}}} = \frac{1}{k_2q_{\text{e,cal}}^2} + \frac{1}{q_{\text{e,cal}}}t \) where k_2 and q_{e,cal} are the pseudo second-order rate constant (g mg⁻¹ min⁻¹) and the estimated amount of absorbed azo dyes (mg g⁻¹).

The surface chemical compositions and chemical states of GO and AQS@rGO were also analyzed by XPS (Figure 1b). The thermal gravimetric analysis (TGA) of the samples were determined by TGA measurement from room temperature to 700 °C in a nitrogen atmosphere (Figure 1c). Pure AQS shows obvious weight loss before 200 °C and between 460 and 500 °C, which is due to the removal of adsorbed moisture and the carbonization of AQS molecules, respectively. The slow weight loss after 500 °C is due to the gradual elimination of sulfur- and oxygen-containing functionalities of the AQS-derived carbon. The same weight decreases between 460 and 500 °C in the AQS@rGO sample clearly verified the attachment of AQS on the rGO matrix. The TEM images of the as-prepared AQS@rGO showed an irregular, thin, and wrinkled film structure with unobvious aggregation (Figure 1d,e), which is similar to some previous studies. Moreover, as shown in Figure 1f, the C 1s, O 1s, and S 1s elements were identified in the electron energy loss spectroscopy (EELS) mapping pictures of the as-prepared AQS@rGO. The EELS results suggested that the functional groups containing oxygen, carbon, and sulfur were uniformly distributed on the AQS@rGO surface. This might further confirm that the AQS was successfully grafted and uniformly covered on the rGO sheet.

2.2. Different Adsorption Capacities of the GO and AQS@rGO. The GO exhibited considerable adsorption capacities toward the test anionic azo dyes, and the calculated parameters of the adsorption isotherms are summarized in Table 1. The calculated correlation coefficients (R²) of the Freundlich model were significantly higher than the ones fitted by the Langmuir model. Furthermore, taking the adsorption of MO by GO as an example (Figure 2), the q_m and the non-linear fitting curve of the Freundlich model (red bar line) for simulating the experimental data of MO adsorption are much closer than that of the Langmuir model (blue dot line). The results indicated that the adsorption of azo dyes by GO belonged to multilayer adsorptions. Additionally, it is observed that the values of “1/n” are smaller than 1, which indicated that the azo dyes were favorably adsorbed onto GO. The kinetic parameters and correlation coefficients (R²) are listed in Table 1, all the adsorption processes were better fitted by the pseudo-second-order kinetic model with higher R² values, and the calculated values (q_{e,cal}) were close to the experimental values. The adsorption behaviors of azo dyes by the as-prepared GO in this study was similar to other GO-based nanoparticles, e.g., the adsorption of MO by GO hydroxide, the adsorption of Congo red (CR) by the graphene oxide-doped nano-hydroxyapatite, and the adsorp-
tion of CR by the cetyltrimethylammonium bromide–graphene composite. 21

The maximum adsorption capacities (q_{max}) of the GO obtained in this study were similar to the pure GO but significantly lower than some modified GO-based materials. 14,15,21 This may be due to that the GO surface lacks active sites (Table S1); (2) the AQS molecules immobilized on GO is mainly accomplished by the noncovalent π–π stacking,19 which therefore reduce the available drive force of interaction between the dye molecules and AQS@rGO; and (3) all the adsorption experiments were conducted in the salt-containing decolorization medium, and the electrostatic interaction between the anion azo dyes and negative surface charges of AQS@rGO was becoming rather weak under the neutral condition. 14

2.3. Enhancement on Biotic Decolorization by AQS@rGO. Bacillus sp. exhibited quinone respiration capacity and decolorization ability under salt conditions in the previous study. 14 As shown in Figure 3, batch experiments with dead cells, GO, and AQS@rGO were separately performed to evaluate their adsorption capacities toward MO dye. Like the adsorption tests, the removal efficiency of MO in the presence of AQS@rGO was less than 10% during the 48 h of incubation time, while the removal efficiency of MO in the presence of GO was achieved at as high as 80%. The apparent degradation rates (k_1) of the batch experiment with a live cell and GO was calculated as 0.356 h^{-1} (R^2 = 0.949), but the enhancement on biotic enhancement in the presence of GO was weak because the adsorption contributes more than 90% of the total removal efficiency (see the cyan closed squares and yellow open squares in Figure 3). On the other hand, the enhancement on degradation by AQS@rGO was biotic, and the decolorization process was followed by first-order kinetics with a high k_2 value of 0.473 h^{-1} (R^2 = 0.999). Compared to the magnetic humic acid (MHA) nanoparticles,4,14 the as-prepared material showed an advantage on the decolorization of MO. Moreover, the preparation process is simple, i.e., one step hydrothermal method and no addition of chemical compounds, e.g., dichloromethane and diethylenetriamine. 22

Experiments under various salt concentrations were performed to evaluate the catalytic stability of AQS@rGO on the decolorization of MO. As shown in Figure 4a, the k_1 value was 0.068 h^{-1} (R^2 = 0.992) with only a live cell under 50 g L^{-1} of NaCl condition, and the k_1 value increased to 0.335 h^{-1} (R^2 = 0.984) when the AQS@rGO was added. The five times increase of acceleration indicates the excellent catalytic effect on the extracellular electron transfer. Analogously, in the batch experiments with 100 g L^{-1} of NaCl (Figure 4b), the acceleration rate was also increased by five times, i.e., the k_1 value increased from 0.092 h^{-1} (R^2 = 0.952) to 0.527 h^{-1} (R^2 = 0.979). Although the experimental data cannot be described by first-order kinetics in the batch experiments with 150 g L^{-1} of NaCl (data not shown), it can be seen clearly in Figure 4c that the decolorization efficiency was significantly improved by the AQS@rGO. The results implied that the as-prepared AQS@rGO could be used as an electron mediator and exhibited good catalytic stability in a salt range of 0 to 150 g L^{-1}. Voltammetric behavior was used to explore the redox characteristics of the solid material,11,22 however, whose effects in the liquid cannot be really reflected due to the measure step in which the solid material has to be fixed onto the electrodes. In this study, we have successfully captured the EPR signal of the semi-quinone radical in the degradation medium in the presence of AQS@rGO (Figure 4d,e). The results directly confirmed that the C=O, i.e., in the form of a quinoid...
structure, may act as semi-quinone radicals and undergoing redox cycling and further increase the electron transfer. 5

2.4. The Application of Recycled AQS@rGO on Decolorization. Two anaerobic sequencing batch reactors (AnSBRs) were performed to investigate the effect of AQS@rGO on biotic reduction of azo dyes under salt conditions. The AnSBR in the presence of GO and AQS@rGO were named R1 and R2, respectively. As shown in Figure S, the first-order kinetic in several treating periods, the degradation efficiency (DE, %) and degradation rate (DR, mol L⁻¹ h⁻¹) were used to compare the treating efficiencies of the two reactors. It can be seen in Table 2 that both the DE and DR values of R2 were improved approximately 1.5–2 times than the ones obtained in R1, which suggested that the addition of AQS@rGO maintains a better catalytic effect than GO. The average DE values in R1 achieved in this study are comparable to those of the continuous reactors with continued addition of free quinones. 5,26 By considering the operation cost of free quinones, the nanocomposite showed a great potential in industrial applications. Moreover, the k values were higher than that obtained by the addition of MHA in our previous study, 4 and the enhancement times of DRs were larger than the reactors feeding with free and/or immobilized humic acid. 27,28 Although the k values of R2 ranged in 0.155–0.35 h⁻¹, which is lower than the ones varied in the range of 0.875 to 1.88 h⁻¹ by modified activated carbon fiber (ACF), 29 the as-prepared AQS@rGO shows a greater competitiveness than ACF because the large specific area and pore volume of ACF make the abiotic adsorption contribute a lot to the apparent degradation rates.

2.5. Proposed Mechanism of MO Reduction in the Presence of AQS@rGO. It was clear from the bioreduction experiments that the formation of redox cycling on AQS@rGO was a key step on the reduction of MO dye. By comparing the FTIR spectra (Figure 1a) and XPS of GO (Figure 1b and Figure S1), the C==O stretching vibrations, i.e., quinones of AQS@rGO, was decreased, but the enhancement on MO reduction by AQS@rGO was significantly higher than that by GO (Figures 3 and S). It is plausible to assume that the C==O group might serve as redox functional groups, but the number was not the only determinant for improving the electron transfer during the redox recycling. It is generally considered that the formation of hydroquinone was the step-limiting process during the biotic process. 34 Reduction of free quinones were usually easy and fast during the extracellular electron transfer (EET) by quinone respiration bacteria through the direct contact model. However, the solid form of quinones was relatively slow and difficult to be reduced because the EET was unspecific. 30 Thus, it can be concluded that the reduction rate of the quinone structure on insoluble RM will be largely increased if the contact opportunity and electron transfer efficiency is further increased.

GO may serve as a high conductive sheet to enable efficient electron transport, which has been confirmed as a great potential for acting as a conductive support due to its good electronic conductivity and large specific surface area. 19 The improvement on decolorization was possibly due to that the redox reaction of C==O on the AQS molecule proceeded more effectively by the aid of the excellent conductive sheet. Moreover, the presence of semi-quinone radicals on solid materials has been detected in the dispersed AQS@rGO solution (Figure 4d,e) until now and that were demonstrated.

Table 2. Calculated Values of the First-Order Degradation Rate and/or Degradation Efficiencies in the Anaerobic Sequencing Batch Reactors

| parameter | R1 | R2 | R1 | R2 | R1 | R2 | R1 | R2 | R1 | R2 | R1 | R2 | R1 | R2 |
|-----------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| DE (%)  | 94.96 | 96.7 | 68.65 | 100 | 59.42 | 74.45 | 59.08 | 100 | 45.53 | 88.99 | 49.92 | 99.43 | 67.45 | 86.41 |
| DR (mol L⁻¹ h⁻¹) | 0.018 | 0.018 | 0.009 | 0.014 | 0.004 | 0.005 | 0.011 | 0.011 | 0.011 | 0.011 | 0.007 | 0.007 | 0.014 | 0.014 |
| k (h⁻¹) | 0.604 | 0.234 | 0.095 | 0.159 | 0.030 | 0.050 | NA | NA | NA | 0.281 | NA | 0.350 | NA | 0.155 |
| R² | 0.973 | 0.981 | 0.937 | 0.965 | 0.683 | 0.922 | NA | NA | NA | 0.999 | NA | 0.989 | NA | 0.865 |

**The treatment time contains seven cycles: cycle I: 0–10.5 h; cycle II: 10.5–25 h; cycle III: 25–56 h; cycle IV: 56–68 h; cycle V: 68–76 h; cycle VI: 76–90 h; cycle VII: 90–102 h. R1 is the bioreactor in the presence of GO, and R2 means the bioreactor in the presence of AQS@rGO. NA means cannot be simulated. ²DE (%) = (1 − C₅/C₀) × 100% where DE means the degradation efficiency of MO in each treating period and C₀ and C₅ are the MO concentrations (mol L⁻¹) in the effluent and influent, respectively. ³DR (mol L⁻¹ h⁻¹) = (C₀ − C₅)/t, and t is the degradation time (h) in each treating period. ⁴k is the pseudo first-order constant (h⁻¹), which was calculated by non-linear fitting of the equation C = C₀e⁻kt. C₀ and C are the initial MO concentration (mg L⁻¹) and MO concentration (mg L⁻¹) at time t (h), respectively.
to be as an effective redox mediator on bioreduction of azo dyes (Figures 3–5). In Figure 6, a model was proposed for the
electron transfer during biotic decolorization of MO in the presence of AQS@rGO. There are two electron transfer pathways in this biochemical system. In pathway I (green dot line), the e− was first generated during substrate oxidation (step 1) followed by the unspecified reduction of MO (step 2). In pathway II (red dot line), the generated e− may quickly transfer through rGO sheets (step 3) followed by the e− transfer from the rGO sheet to C═O (step 4). In the electrochemical system, the AQS offers strong adhesion to the rGO surface via strong π–π interactions, which induces a large charge redistribution and the formation of a space-charge layer, and the latter one facilitates the efficient charge injection from the highly conductive rGO sheets to the C═O of the AQS molecule. Therefore, in this bioelectrochemical process, the reducing equivalents, i.e., e− was more likely transferred from the rGO to C═O in a similar way. After accepting the e−, the hydroquinones of AQS molecules were formed (step 4), and then the formed hydroquinone may chemically reduce the azo dye rapidly (step 5).

3. CONCLUSIONS

The as-prepared AQS@rGO nanoparticle was demonstrated to be an efficient solid RM to improve the decolorization of azo dye. The nanoparticle exhibited rather weak adsorption capacity toward the azo dye, but the catalytic activity of the biotic reduction was improved. Approximately 1.5–2.5 folds of improvement on k values were achieved with AQS@rGO when compared with those with GO. Moreover, the as-prepared AQS@rGO maintained a sustainable and efficient catalytic effect on the biotic reduction of MO in AnSBR during the seven operation periods. The enhancement on reduction of azo dye was possibly due to that the redox ability of the C═O on the AQS molecule acted more effectively with the aid of the excellent conductive sheet. In more detail, the rGO sheets and AQS molecule easily form a space-charge layer by π–π interactions, which facilitates an efficient e− transfer from the highly conductive rGO sheets to the C═O of the AQS molecule.

4. METHODS AND MATERIALS

4.1. Chemicals. Graphite powder (G103919) and AQS (S107065) were bought from Aladdin. Azo dyes, i.e., methyl orange (MO, Dye content 85%), reactive black 5 (RB5, dye content 50%), and acid blue 113 (AB113, dye content 50%) were ordered from Sigma-Aldrich without further purification. LIVE/DEAD BacLight Bacterial Viability Kit (L13152) was purchased from ThermoFisher. The purities of other chemical reagents used are all analytical grade, and all of them were ordered from Sinopharm Chemical Reagent, China.

4.2. Microorganism and Culture Conditions. Strain Bacillus sp. (save number: CICC 23870) was chosen as the model strain, which was routinely cultured in a Luria-Bertani (LB) medium. The main constituents of the LB was listed as follows: 10 g L−1 of tryptone, 5 g L−1 of yeast extract, 15 g L−1 of Agar, and 10 g L−1 of NaCl; the pH was adjusted to 7.2. Biodecolorization was performed in a salt-containing medium containing the following constituents: 2.5% (v/v) of an SL-6 trace element, 6 g L−1 of glucose, 6 g L−1 of Na2HPO4·12H2O, 0.24 g L−1 of KH2PO4, 1 g L−1 of NH4Cl, 0.5 g L−1 of NaCl, 0.003 g L−1 of CaCl2, 0.24 g L−1 of MgSO4·7H2O, and 50 g L−1 of NaCl. After azo dyes were prepared in predetermined concentration, the pH value of the salt-containing medium was also adjusted to 7.2. The SL-6 trace element was referred to our previous study. To avoid the precipitation of salts during sterilization in an autoclave, glucose, and MgSO4 were prepared in a solution and autoclaved separately, and then the solutions were combined sterilely after they were cooled in an aseptic operating board.

4.3. Preparation of the AQS@rGO Nanoparticles. Modified Hummers’ method was employed to produce graphene oxide nanosheets from natural graphite powder. Then, the AQS@rGO nanoparticles were prepared by the one-step hydrothermal method. Briefly, 60 mL of GO solution (2 g L−1) was first mixed with 0.24 g of AQS and stirred for 6 h at room temperature. Then, the mixture was transferred into a 100 mL polytetrafluoroethylene-lined stainless-steel autoclave and heated for 12 h at 180 °C. The autoclave was cooled to obtain the AQS@rGO hydrogel, which was then thoroughly washed by high pure water for three times and lyophilized to obtain the AQS@rGO powder.

4.4. Adsorption of Azo Dyes. To evaluate the adsorption capacities of the as-prepared materials, different initial concentrations of the azo dyes (C0, mg L−1) were separately added into 50 mL of the salt-containing decolorization medium with 25 mg of adsorbents. The mixture was shaken at 30 °C with a rotation speed of 120 rpm for 48 h. Liquid samples were separated by centrifugation and filtration, and then the supernatant was carefully removed after they were cooled in an aseptic operating board.

4.5. Biotic Reduction of MO under Salt Condition. Strain Bacillus sp. was first aerobically growth in an LB medium at 35 °C till the OD660 value (optical density at 660 nm) of the cell solution reached around 1.5. Cells were collected by freezing centrifugation at 8000 × g for 15 min, and the cell sediment were softly washed by phosphate buffer solution (PBS, 50 mM, pH = 7.2) for three times. The cleaned cells
were then injected into the oxygen-free salt-containing decolorization medium in the sterile operating table, and the final cell concentration was adjusted to approximately 0.6 g of cell dry weight (CDW) per liter. To evaluate the effect of the as-prepared materials on biotic decolorization, AQS@rGO was added to the salt-containing decolorization medium, and the final concentration was set at 1 g L\(^{-1}\) except other statements.

4.6. Analytical Methods. The analytical methods were provided in the Supporting Information.

**ASSOCIATED CONTENT**

Supporting Information
The Supporting Information is available free of charge at [https://pubs.acs.org/doi/10.1021/acsomega.0c02837](https://pubs.acs.org/doi/10.1021/acsomega.0c02837).

Analytical Methods, component analyses of GO and AQS@rGO; high-resolution XPS spectra of Cls, O1s, and S2p of GO and AQS@rGO; and adsorption of AB113 (a), RB5 (b), and MO (c) onto the AQS@rGO at different initial dye concentrations (PDF)

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Notes
The authors declare no competing financial interest.

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