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Graphene oxide composite membrane accelerates organic pollutant degradation by *Shewanella* bacteria

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ABSTRACT

Degradation of various organic pollutants by bacteria has been proved to be an economical and environmentally friendly method. The key challenge in making these technologies widely available is their low degradation efficiency. Here, we report a composite membrane composed of graphene oxide and polyvinyl alcohol (GO-PVA) which can markedly enhance the efficiency and rate of *Shewanella* bacteria to degrade Congo red (CR). The degradation efficiency of *Shewanella* bacteria alone on CR solution was about 42% at 72 h. After the addition of the GO-PVA membrane, the degradation efficiency reached 91% at the degradation time at 36 h. The degradation efficiency of CR was increased by two times and the degradation rate was increased by four times in the presence of GO-PVA membrane and *Shewanella* bacteria compared to *Shewanella* bacteria alone. This indicates that the CR could be rapidly and completely degraded by increasing the input amounts of GO-PVA membrane in the process of *Shewanella algae* degradation of CR. Moreover, the GO-PVA membrane showed good recyclability and reusability. The biocatalysts performance of the GO-PVA membrane did not decrease after 10 cycles. Furthermore, the role of the GO-PVA membrane in the degradation process and the degradation products of CR were discussed.

Key words: Congo red degradation, efficiency enhance, graphene oxide, recyclability, *Shewanella* bacteria

HIGHLIGHTS

- The efficiency of *Shewanella* bacteria to degrade CR is about 42% at 72 h.
- The GO-PVA can significantly improve the degradation rate and efficiency of CR.
- The degradation rate was increased by four times and the efficiency was increased by about two times.
- The GO in the GO-PVA plays as a redox mediator in the degradation of CR.
- The GO-PVA shows good recyclability and reusability.

INTRODUCTION

Organic dyes are widely used in various industrial fields such as textiles, cosmetics, plastics, leather, and paper. Among organic dyes, azo dyes account for more than 60% of the dye output because of their excellent dyeing ability, which also has made it become one of the serious organic pollutants in industrial wastewater (Shah 2014). Upon release into the environment, azo compounds are mutagenic and carcinogenic to humans (Pazdzior *et al.* 2017). Traditionally, azo dyes in wastewater are physically retrieved by adsorption (Ben Jeddou *et al.* 2021; Khalaf *et al.* 2021), precipitation, filtration, and reverse osmosis to retrieve them, but are of high cost and ineffective (Wang *et al.* 2018). In addition, the azo dye can also chemically be degraded by ozonation, advanced oxidation processes (Aziz *et al.* 2018; Aziz 2019), Fenton's reagent, photocatalysis (Qu *et al.* 2021), and electrochemistry, unfortunately producing problems of high energy consumption, secondary

pollution, and economic and technical difficulties (Imran *et al.* 2015). Thereby, the effective treatment of azo dye pollutants is still a challenging task for environmental scientists.

In contrast with physical and chemical treatments, some natural bacteria have demonstrated their excellent capability to degrade various organic pollutants, and are recognized as an economic and green approach due to less energy consumption and secondary pollution (Sarkar *et al.* 2017). Typically, *Shewanella* can produce abundant azo reductase, which can catalyze the reduction of double bonds of azo molecules and decompose pollutants into low-toxic products under aerobic conditions (Fredrickson *et al.* 2008). Although people have tried to change the culture conditions such as oxygen content, carbon source, temperature, and pH to achieve better degradation effects, the degradation efficiency is too low to meet the requirements of practical application (Cao *et al.* 2017). The recent advance is the addition of redox mediator (RM) molecules as 2-anthraquinone sulfonate (AQS), speeding up the electron transmission between reductase and azo dyes to enhance the degrading efficiency up to 95% (Huang *et al.* 2017). However, most of the mediators themselves are harmful to the environment, and small molecules of RM can cause secondary pollution with sewage loss, which greatly limits their application. Therefore, it is very important to develop new biophilic material with good catalytic performance and recyclability for the treatment of dye wastewater.

Graphene oxide (GO) is a kind of environment-friendly versatile material with a planner structure that contains rich hydroxyl groups, carbonyl and epoxy groups at its base plane, and carboxyl groups at its lateral edges (Lerf *et al.* 1998), not only providing a strong absorbing affinity to various organic molecules (Fakhri *et al.* 2017) and metal ions (Wei *et al.* 2018) but also enabling the bacteria to easily immobilize at its large surface due to good biocompatibility (Wang *et al.* 2011). In addition, PVA with hydroxyl groups can further improve the interactions with these pollutants and bacteria. Conversely, GO nanosheets possess a large π-conjugated structure that is especially effective in absorbing dyes such as azo compounds by π−π interactions (Shen *et al.* 2010). It is worth noting that, in the case of degrading organic and metal pollutants, the π-conjugated structure can play a key role of electron transfer intermediator role between pollutants and catalysts (Choi *et al.* 2011), enzymes, or bacteria (Shen *et al.* 2010). Moreover, the addition of PVA can also fix GO particles to avoid the secondary pollution caused by GO with the loss of wastewater.

In this study, we prepared a GO-PVA composite membranes by mixing GO and PVA solution. GO provided abundant reaction sites to promote *Shewanella* degradation of Congo red (CR), while the addition of PVA could immobilize GO and increase the biocompatibility of GO to *Shewanella*. Herein, we conducted a variety of experiments to evaluate the positive performance of GO-PVA composite membrane for *Shewanella* degradation of CR. In addition, the possible removal sequence and changing state of functional groups of CR during microbial degradation was proposed. The research results are helpful for people to better understand the mechanism of *Shewanella* degradation of CR dye and its application in dye wastewater bioremediation, and provide a reference for the application of graphene materials in wastewater biological treatment.

MATERIALS AND METHODS

Chemical reagents and bacterial strains

Graphite flakes (5,000 mesh, CP), polyvinyl alcohol 1,788 (PVA1788, 99%), sulfuric acid (H_2SO_4, mass fraction −98%), potassium permanganate ($KMnO_4$), hydrochloric acid (HCl), hydrogen peroxide (H_2O_2, 30%), all the above reagents were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Unless otherwise specified, all other reagents used above are of analytical grade. Congo red (98%) was purchased from Aladdin Biochemical Technology Co., Ltd (Shanghai, China).

Shewanella algae (MCCC 1A02601) was obtained from the Marine Culture Collection of China (Xiamen, China). They were maintained and grown in Tryptone Soya Agar (TSA). The degradation studies were performed in Tryptic Soy Broth (TSB). Both TSA and TSB were purchased from Haibo Mall (Qingdao, China).

Preparation of GO and GO-PVA composite membrane

GO sheets were prepared by oxidation and exfoliation of graphite flakes according to previously published literature (Zhang *et al.* 2012). The specific preparation process is as follows: 1.0 g graphite powder (5,000 mesh) and 48.0 mL H_2SO_4 (mass fraction 98%) are transferred into a three-necked flask (500 mL) and stirred at 1,500 rpm/min for 3.5 h. The reaction temperature was kept below 5 °C in an ice bath, and then 6.0 g $KMnO_4$ was slowly added and the mixture stirred for 4 h in an ice bath. After that, the temperature was raised to 35 °C and the mixture stirred for 2 h. Then 40 mL of water was slowly added dropwise to the above solution (being careful of violent exothermic acid mist). The mixture was heated to 95 °C, stirred for 30 min, then 100 mL of water was added, and immediately followed by the addition of 10 mL of H_2O_2 (30%, beware of

large bubbles, which may overflow). The color of the solution turned golden yellow. The solution was centrifuged and the precipitate retained. The precipitate was washed with 10% HCl three times, then washed two times with deionized water, and freeze-dried for 48 h.

The dried GO powder was weighed and dissolved in deionized water to prepare a GO solution of 2.00 g/L and then sonicated in an ultrasonic cleaner for 6–8 h to peel off the stacked graphene oxide sheets. Next, 4.00 g PVA1788 pellets was weighed out, 500 mL water was added, the mxture was heated to 90 °C, stirred at 1,000 rpm/min for 2 h, and then cooled to room temperature to prepare an 8.00 g/L PVA solution for later use. A graduated cylinder was used to measure the specific volume ratio of the GO and PVA1788 solution in the flask. The solutions were heated to 90 °C and stirred at 1,000 rpm for 2 h, and cooled to room temperature. Finally, the GO-PVA mixed solution was dried in a blast drying box to form a GO-PVA membrane. We prepared different ratios of GO-PVA membranes by mixing specific volume ratios of GO sheet solution and PVA solution at 1:1, 1:2, 1:4, 2:1, and 4:1, respectively. The resulting membranes are defined as GO-PVA$_{1-1}$, GO-PVA$_{1-2}$, GO-PVA$_{1-4}$, GO-PVA$_{2-1}$, and GO-PVA$_{4-1}$.

Congo red degradation experiments

First, the purchased freeze-dried powder of *Shewanella* algae was inoculated onto TSA and passaged twice to ensure that it was fully rejuvenated. After inoculation in 78 mL TSB medium, the aerobic culture made the initial cell mass $OD_{595} = 0.3$. Figure S2 (Supplementary Material) plots the growth curve of *Shewanella* algae. Then 2.00 mL 4.00 g/L CR solution was added and the final CR concentration in the culture solution was calculated as 100 mg/L. To study the effect of GO-PVA composite membrane on the *Shewanella* degradation of CR, 8 mg GO-PVA$_{4-1}$, GO-PVA$_{2-1}$, GO-PVA$_{1-1}$, GO-PVA$_{1-2}$, and GO-PVA$_{1-4}$ composite membrane were added. The control group was the degradation experiment of CR by *Shewanella* alone.

During the experiment, unless otherwise specified, the mass of the GO-PVA membrane was 8 mg while the culture medium (CR concentration: 100 mg/L) was 80 mL. Under aerobic conditions, all biological systems were reacted in a wide-mouthed conical flask (500 mL) placed on a shaker (30 °C, 100 rpm/min). Here, 1 mL aliquots were taken out at different degradation times at 0, 2, 4, 6, 8, 10, 12, 24, 36, 48, 60, and 72 h, respectively, and centrifuged at 8,000 rpm/min for 6 min to remove cells, culture medium, etc. Next, 100.0 μL of the supernatant was transferred on a 96-well plate to measure the absorbance at 492 nm using a microplate reader. The above experiment was repeated at least three times.

Analytical methods

The concentration of CR was determined by measuring the absorbance of the solution at 492 nm using a microplate reader (Molecular Devices, EMax Plus, USA). The standard curve for the CR concentration and absorbance is shown in Figure S3. The degradation efficiency (Asses *et al.* 2018) was calculated according to the following formula:

$$\text{Degradation efficiency (\%)} = \frac{(A_0 - A_t)}{A_t} \times 100\%$$

where A_0 refers to the absorbance of the supernatant at 492 nm when the fade time is 0 h; A_t refers to the absorbance of the supernatant at 492 nm when the fade time is t h.

To reveal the degradation mechanism, UV-visible spectrophotometer (Shimadzu UV-1900i Japan), Fourier transform infrared spectroscopy (Thermo Scientific, iS50R FT-IR, USA), the Quadrupole Time-of-Flight mass spectrometer (Agilent, G6500, USA) analyzed the degradation products of CR. Besides, we also used a field emission scanning electron microscope (TESCAN, VEGA3, Czech Republic) and a field emission transmission electron microscope (JEOL, JEM-2100F, Japan) to estimate the morphology of GO and GO-PVA, and a laser microscopic Raman spectrometer (Thermo Scientific, DXR, USA) study the chemical properties of GO-PVA and rGO-PVA membranes.

RESULTS AND DISCUSSION

Degradation mechanism of the GO-PVA membrane with *Shewanella* algae to azo dye

The specific mechanism of bacterial degradation of azo dyes is very complex, but there are mainly three degradation types. One is intracellular degradation, the other is degradation on the cell membrane, and the third is electron transfer through redox intermediates. *Shewanella* bacteria can transfer electrons from a cell interior to external electron acceptors (Myers & Nealson 1988). Here, we proposed to employ the GO as an RM to enhance the speed of electron transfer among bacteria

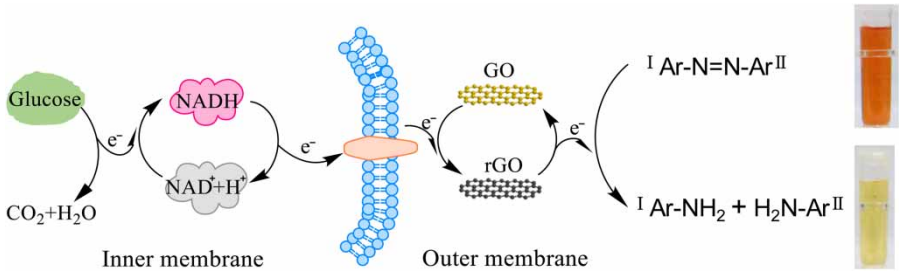

Figure 1 | Proposed mechanism of GO-PVA membrane promoting *Shewanella* degradation of azo dyes.

and GO and azo dyes, thus improving the degradation efficiency of azo dyes. The possible degrading mechanism of the GO-PVA membrane with *Shewanella* algae to azo dye is demonstrated in Figure 1. First, the bacteria oxidize nutrients such as glucose to obtain electrons and transfer the electrons to the cell membrane through intracellular respiratory enzymes (such as NADP). In the degradation process, most of the bacteria adhered to the surface of the GO-PVA membrane due to the good biocompatibility of GO and PVA. The electrons produced by the bacteria are enriched on the surface of the GO-PVA membrane. Some electrons are accepted by the azo dyes, in addition more electrons were accepted by GO. After accepting the electrons, oxygen-containing functional groups such as carbonyl and epoxy groups in GO are reduced to hydroxyl groups, which are then isomerized into phenols with the aromatic rings in GO (Wang *et al.* 2011; Zhao *et al.* 2018). At this time, GO is reduced to rGO by bacteria. After that, the phenolic structure in rGO reduces CR on the GO-PVA film, so that the azo bond of CR is reduced and broken into an amino group. In addition, the reduction of azo dyes also causes rGO to be oxidized into GO, and the cycle repeats. Thus, the process of degradation of azo dyes is achieved. GO acts as an electron transfer intermediator between *Shewanella* and of azo dyes in the process of degradation azo dyes which will speed up the electron transmission between *Shewanella* and azo dyes to enhance the degradation rate and efficiency.

Characterizations of GO and GO-PVA membrane

The GO prepared by the modified Hummers method was dispersed by ultrasound for 6–8 h, making the GO lamellae thinner, which was conducive to the growth of bacteria, the adhesion of CR, and the exposure of the reaction site. The SEM and TEM images (Figure S4A-B) of GO show that the synthesized GO is mostly less than 10 layers. The prepared GO-PVA composite membranes have a smooth surface and the thickness is about 10–15 μm, as shown in Figure 2(a). The inset shows a fault in the membrane with distinct layered structures visible. Also, with the increasing amounts of GO, the color of the GO-PVA membranes changes from reddish-brown (GO-PVA$_{1-4}$) to black (GO-PVA$_{4-1}$) (Figure S4C-D). The fluidity and hydrophilicity of PVA make GO sheets disperse evenly in membranes and increase the affinity with *Shewanella* bacteria.

The GO-PVA membranes were immersed into the *Shewanella* bacteria solution and CR solution for 1 h. Then taken out of the membranes, washed with deionized water several times, and characterized by SEM and Raman spectra. The SEM image

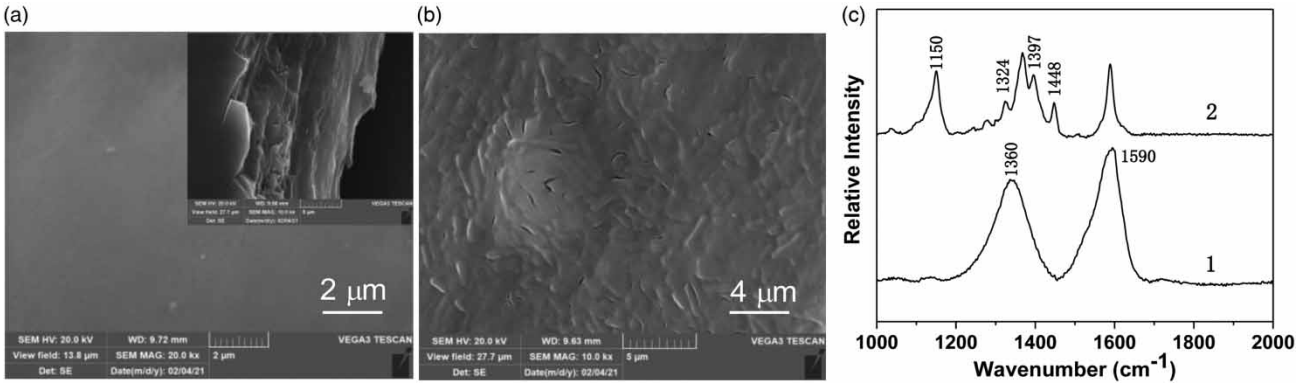

Figure 2 | (a) The SEM images of GO-PVA membrane and (b) after immersion into the *Shewanella* bacteria solution, (c) the Raman spectra of GO-PVA membrane before (1) and after (2) immersion into the CR solution.

shows that *Shewanella* bacteria can rapidly adhere to the surface of GO-PVA membranes as seen in Figure 2(b). The Raman spectra results are shown in Figure 2(c). The two strong peaks at 1,360 and 1,590 cm^{-1} (line 1) are the characteristic D and G peaks of GO, respectively. After immersion into the CR solution, many CR molecules were adsorbed onto the surface of the membrane. The Raman result (line 2) shows that the N = N stretching vibration peak is at 1,448 cm^{-1}, the C-NH_2 symmetric stretching vibration peak is at 1,397 cm^{-1}, the C-C stretching vibration peak is at 1,324 cm^{-1}, and the stretching vibration peak at 1,150 cm^{-1} is the stretching vibration peak of aromatic C-S. These results confirmed that the affinity of the membrane to bacteria and CR molecules could enrich the bacteria and CR molecules close to the surface of the GO-PVA membrane. The shorter distance would enhance the speed of electron transfer among bacteria and GO and azo dyes, thus the efficiency of degradation of azo dyes by *Shewanella* bacteria would be improved.

Degrading CR by *Shewanella* algae and GO-PVA membranes

To investigate the effects of different amounts of GO in the degradation process under *Shewanella* algae, we prepared different ratios of GO-PVA membranes named GO-PVA_{1-1}, GO-PVA_{1-2}, GO-PVA_{1-4}, GO-PVA_{2-1}, and GO-PVA_{4-1}. The CR was selected as the degradation target molecules and the concentration of CR is 100 mg/L in this study. The degradation efficiency of CR by different GO-PVA membranes under *Shewanella* algae is illustrated in Figure 3(a). The black line is the degradation process of only *Shewanella* algae in which the CR was approximately degraded by about 42% at 72 h. Compared with the control group, supplemental addition of GO-PVA membranes (GO-PVA_{4-1}, GO- PVA_{2-1}, and GO-PVA_{1-1}) could significantly increase the degradation rate and efficiency of CR during the initial degradation process. Especially, with the existence of GO-PVA_{4-1} and *Shewanella* algae, the degradation efficiency of CR reached up to 95% near complete degradation. The corresponding optical images of GO-PVA_{4-1} and *Shewanella* algae are shown in Figure 3(c). The images show that the color of the CR solution becomes lighter after the addition of GO-PVA_{4-1} compared with the control at 12 h. After 60 h, the color of the solution almost faded, while the control group had little discoloration. This showed that the addition of GO-PVA_{4-1} in the process of *Shewanella* algae degradation of CR can highly improve the degradation rate and efficiency of CR. The improvement in the degradation rate and efficiency of CR is not affected by the physical adsorption of GO-PVA membranes. From Figure 3(b), we found that the addition of GO-PVA membranes had slight effects on the degradation and physical adsorption of CR. Therefore, the presence of the GO-PVA membrane promotes the biodegradation of CR by *Shewanella*.

Compared with the GO-PVA membranes with lower GO content, higher GO content in GO-PVA promoted excellent promotion activity in the process of *Shewanella* algae degradation of CR. This indicated that GO played a key role in the biodegradation process of CR. However, the addition of GO-PVA_{1-2}, GO-PVA_{1-4} membranes reduces the degradation efficiency of the system. Especially in the first few hours, the degradation efficiency is hindered. The reason may be attributed to the high content ratio of PVA in GO-PVA_{1-2} and GO-PVA_{1-4} that is dissolved in the process of degradation. There is a strong hydrogen bond between the dissolved PVA and CR molecules which affects the determination of absorbance (Yahia & Keshk 2017). Therefore, as a control experiment, we added pure GO and pure PVA membrane to the degradation process of

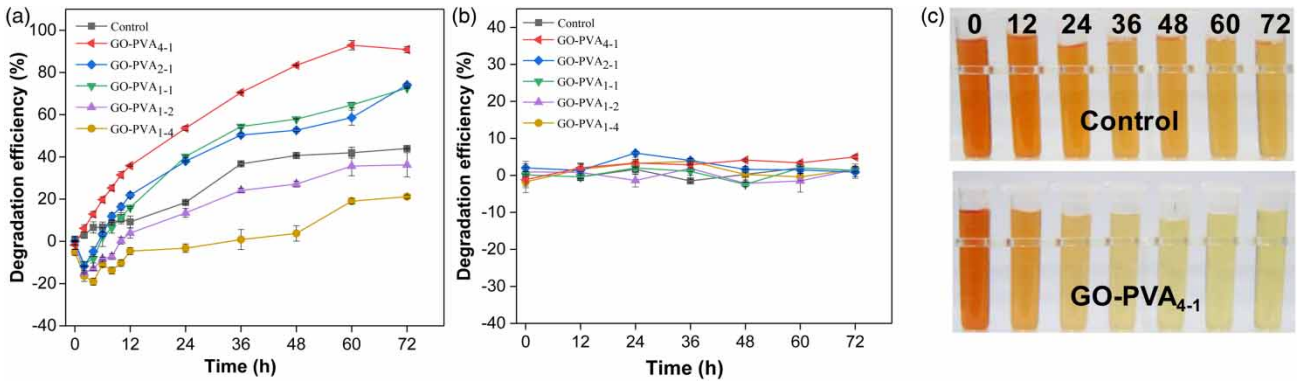

Figure 3 | (a) Degradation of CR by *Shewanella* after addition of different GO-PVA membranes, (b) the adsorption studies of CR on different GO-PVA films, and (c) optical images of CR degradation by *Shewanella* (Control) and the addition of GO-PVA_{4-1} at the different degradation time (0, 12, 24, 36, 48, 60, and 72 h).

Shewanella algae to CR. It was found that the GO alone can also promote the *Shewanella* algae degradation efficiency of CR (increase to 75%), while the PVA alone has a huge inhibitory effect on the *Shewanella* algae degradation of CR (Figure S5A).

However, pure GO and PVA membrane in the solution could dissolve and disperse in solution in 3–5 min. Dispersed GO nanosheets cannot provide a large surface for the attachment point for *Shewanella* algae so that the efficiency of electronic transmission is reduced. Thus, the degradation rate and efficiency of pure GO with *Shewanella* algae to degrade CR (75%) was lower compared with the GO-PVA$_{4-1}$ (95%). In addition, the black colored GO nanosheets greatly affected the absorbance of the CR molecules and caused inaccurate detection results. The strong hydrogen bond between free PVA and CR molecules led to the increasing absorbance of CR so that the degradation efficiency was reduced (Figure S5B). Thus, GO-PVA$_{4-1}$ is the optimal membrane in the degradation process of *Shewanella* algae to CR.

Effects of GO-PVA$_{4-1}$ amount to the degradation of CR

The amount of GO-PVA$_{4-1}$ on the degradation of CR by *Shewanella* algae have been investigated. The recyclability and sustainability of GO-PVA$_{4-1}$ also have been discussed to further evaluate the practical value of GO-PVA$_{4-1}$ in promoting the degradation of *Shewanella* algae to CR. First, we prepared 100 mL of *Shewanella* algae culture medium containing 100 mg/L CR and then supplemented it with 0, 2, 5, 10, 20, 50 mg of GO-PVA$_{4-1}$ to evaluate the degradation rate of CR. From the experimental results in Figure 4(a), even with a trace amount (2 mg) of GO-PVA$_{4-1}$ membrane supplemented (the mass of CR was calculated as 10 mg), the degradation efficiency and rate of CR can be produce a large increase (about 70%). While continuing to increase the quality of supplemental GO-PVA$_{4-1}$, basically its efficiency and speed have been enhanced, especially when the added mass was 50 mg, the rate of bacterial *Shewanella* algae degradation of CR was enhanced about four times, the degradation efficiency was increased up to 95%. This indicates that the CR could be rapidly and completely degraded by increasing the input amounts of GO-PVA$_{4-1}$ in the process of *Shewanella* algae degradation of CR.

To evaluate the potential of the GO-PVA$_{4-1}$ membrane as a recyclable biocatalyst to promote the degradation of CR by *Shewanella* algae, we conducted a series of degradation experiments (10 cycles) through the membrane and then recovered GO-PVA$_{4-1}$ and reused it in the same reaction system. The cycle experiment can be briefly described as follows: after the completion of a CR degradation experiment, the GO-PVA$_{4-1}$ composite membrane was rinsed with deionized water four times to remove bacteria, CR degradation products, metabolic wastes, and other surface impurities, to eliminate their influence on the next cycle experiment, and then the GO-PVA$_{4-1}$ membrane was transferred to a new system that performed the same CR degradation experiment. During the cycling experiment, the composite membrane always used the GO-PVA$_{4-1}$ membrane at the beginning of the cycling experiment, and the variable was only to keep the CR concentration of the new system at 100 mg/L and the $OD_{595} = 0.3$ of the bacteria (the system biomass was the same). In the cycling experiment, the

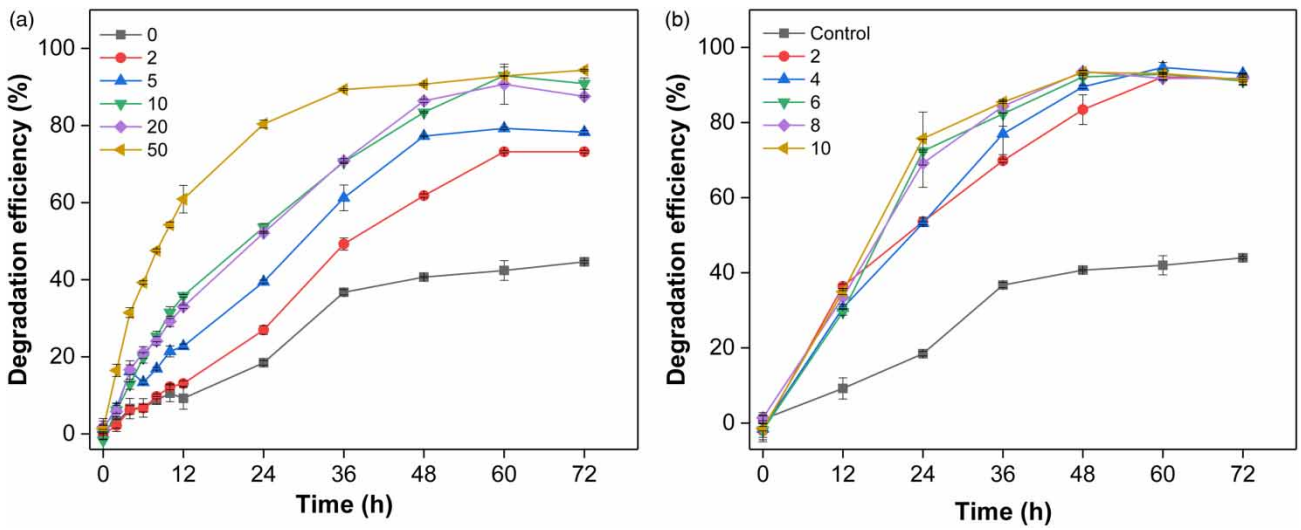

Figure 4 | (a) The influence of GO-PVA$_{4-1}$ amounts (0, 2, 5, 10, 20, 50 mg) on the degradation of CR by *Shewanella* algae, and (b) the cycling performance of GO-PVA$_{4-1}$ membrane on the degradation of CR by *Shewanella* algae in 10 consecutive cycle experiments (2, 4, 6, 8, 10 indicate the number of cycles of the film).

change of the OD_{492} value of the solution was recorded at each time point of 0, 12, 24, 36, 48, 60, and 72 h to evaluate the catalytic performance of the GO-PVA_{4-1} (Figure 4(b)).

Compared with the control group, the GO-PVA_{4-1} is present in each cycle. Therefore, it was observed that the degradation rate and efficiency of the cycle experiment CR were much higher than that of the control group in the 10 cycles experiment, indicating that the GO-PVA_{4-1} membrane had an ideal biocatalytic performance. In addition, with the increase in the number of cycle experiments, the catalytic performance improved and finally stabilized at the optimal level. The increase in catalytic efficiency may be due to the continuous progress of the cycling experiment, which further reduced the GO in the GO-PVA_{4-1} membrane, reducing the reaction time of the step of reducing the GO by the bacteria. For the question of how GO promotes the degradation of CR of *Shewanella*, it was necessary to construct an experiment protocol with *Shewanella* mutant strains for further study (Jiao *et al.* 2011; Cai *et al.* 2012; Li *et al.* 2020). Also, in the 10-cycle experiments, the wet GO-PVA_{4-1} membrane showed a certain degree of toughness (180° folded in half without cracking), which was completely different from bending 90° when dry. The mass range of the GO-PVA_{4-1} membrane after the cycling experiment was 98% ±4%, indicating the stability of the membrane. In the cycling experiment of promoting *Shewanella* degradation of CR by the GO-PVA_{4-1} membrane, the catalytic performance of the membrane did not decrease and the physical properties did not change dramatically. These results indicated that the GO-PVA_{4-1} membrane has a very good cyclic biocatalytic function.

Verification of GO-PVA functions in the degradation process

After the degradation process is completed, the GO-PVA membranes which participated in the *Shewanella* algae degradation process of azo dyes were taken out and thoroughly cleaned with deionized water, and dried. Then they were characterized by Raman spectroscopy and FTIR. Two representative peaks (located at −1,360 and −1,590 cm^{-1}) generated by the D-band and G-band peaks, respectively, appeared in the Raman spectrum of the GO sheet. In this study, the Raman intensity ratio of D-band and G-band peaks (I_D/I_G) were usually used as an estimate of disordered graphite indicators (Bansal *et al.* 2015; Parra *et al.* 2015). As shown in Figure 5(a), the GO-PVA_{4-1} membrane recovered from the degradation experiment showed typical Raman spectra of carbonaceous materials and had a higher I_D/I_G ratio (≈0.9), indicating the generated rGO-PVA composite that showed higher graphite order than the original GO-PVA_{4-1} ($I_D/I_G \approx 0.7$).

In addition, FTIR analysis (Figure 5(b)) also showed that the GO-PVA_{4-1} (the black line below Figure 5(b)) had an -OH stretching vibration at 3,438 cm^{-1}, -C = O stretching vibration at 1,722 cm^{-1}, -OH bending vibration at 1,405 cm^{-1}, and the C-O stretching vibration at 1,085 cm^{-1}. However, compared with GO-PVA, the C-O tensile vibration strength of the degraded rGO-PVA film decreased sharply at 1,085 cm^{-1}. The vibration peak at 1,617 cm^{-1} was due to the vibration absorption of water molecules. This was because the air-drying method of GO cannot completely remove the water between the GO sheets, and even the air-dried GO contains 8% water (Szabó *et al.* 2005). This confirmed that the GO component in the GO-PVA membrane has undergone a microbial modification and reduction process after the degradation experiment (Mehdinia *et al.* 2014; Cobos *et al.* 2018; Wang *et al.* 2018; Camedda *et al.* 2019; Luo *et al.* 2019).

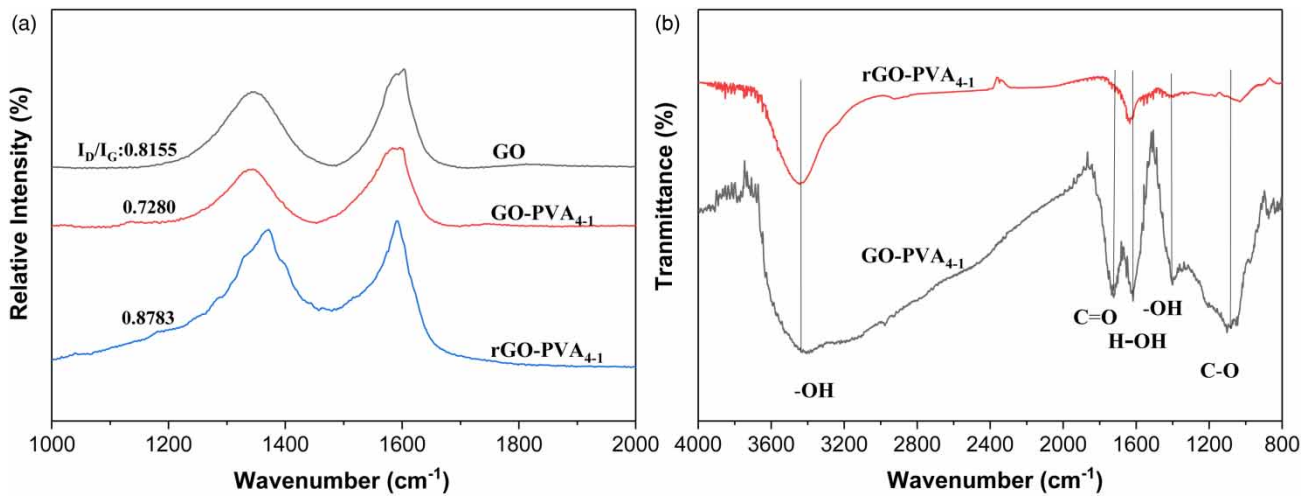

Figure 5 | (a) The Raman spectra of GO, GO-PVA_{4-1}, and rGO-PVA_{4-1}, and (b) FTIR ofGO-PVA_{4-1} and rGO-PVA_{4-1}.

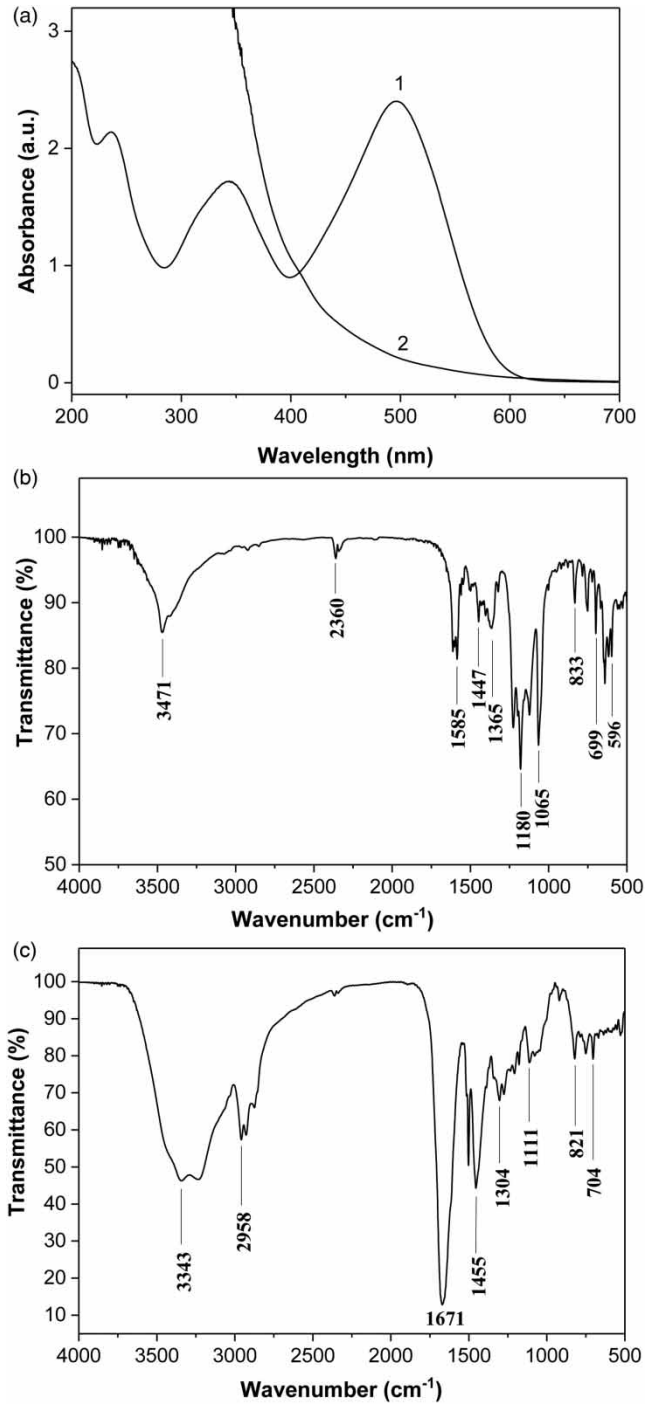

Figure 6 | (a) The UV absorption of the CR solution (1) and 72 h after *Shewanella* algae treatment (2), (b) the FTIR of CR dye, and after 72 h treatment with *Shewanella* algae (c).

Analysis of CR degradation products

The UV-vis spectrum analysis (200–800 nm) of the supernatant before and after the *Shewanella* algae degradation of CR is shown in Figure 6(a). From line (1), the UV absorption spectrum of the 100 mg/L CR solution, it can be ascertained that the three UV absorption peaks of CR were 236.5 nm, 344 nm, and 497.5 nm. From line (2), we found that the absorption peak of CR at 497.5 nm disappeared after 72 h of degradation, and the ultraviolet absorption began to increase sharply in

the wavelength range 450–340 nm. This was because of the n–π*, π–π*, n–σ*, π–σ*, and other electronic transitions caused by aromatic compounds such as biphenyl and naphthalene, generated after the degradation of CR, that increased sharply. It can be shown that CR has been completely degraded by aromatic small molecules by *Shewanella* algae at this time.

To further determine the structure of the degradation products, the FTIR spectroscopy of CR and the degradation products were characterized. As shown in Figure 6(b), the CR spectrum shows the peak for -NH_2, and the N-H bending vibration peak is at 3,471 cm^{-1}. The three weak peaks around 3,000 cm^{-1} are several weak bands caused by overtones and combined vibrations; at 1,612 and 1,585 cm^{-1} and are due to the C = O stretching vibration peaks; the 1,447 cm^{-1} vibration peak is the bending of C-H; at 1,365–1,065 cm^{-1} are the bending, stretching vibration peaks of N-H, C-N; and 850–600 cm^{-1} the characteristic absorption peak of substituted aromatics. The FTIR spectrum of CR degradation products is shown in Figure 6(c). There is a broad and strong peak at 3,343 cm^{-1} due to O-H and N-H stretching vibrations, and a sharp and strong peak at 1,671 cm^{-1} due to C = O bending vibration, which may be caused by the extraction of residual ethyl acetate. At 1,455 cm^{-1}, 1,304 cm^{-1} peaks are caused by O-H bending vibration and -NO_2 stretching vibration, respectively. However, the bending and stretching vibration peaks of N-H, C-N at 1,365–1,065 cm^{-1} were greatly reduced. The FTIR spectrum results show the reduction of aromatic amine peaks -NH_2 and the appearance of new functional group peaks such as -OH and -NO_2. Furthermore CR is completely degraded after the degradation experiment, which is consistent with the results obtained by the UV spectrum consistent.

The ESI-MS spectra of CR (Figure 7(a)) and its degradation products after 72 h of degradation (Figure 7(b)) were also performed to further clarify the structure of the degradation products and explain the degradation mechanism of CR in the presence of GO-PVA. According to the mass spectra results, we have listed the possible chemical structures of the molecular ion peaks/fragment ion peaks of the mass spectra in Table S1 and thus proposed possible pathways for the degradation of CR (Figure S6). Firstly, -N = N- in CR is hydrogenated to -NH-NH-, and then -NH_2 is oxidized to -NO_2. Secondly, the cleavage between NH and NH and the de-SO_3Na group undergoes a race reaction, according to the molecular ion peak 339 of Figure 7(b). This confirms that the de-SO_3Na group is the main reaction. Finally, deamination, amine oxidation, and desulfurization are further carried out to produce intermediates, thereby converting complex dye molecules into simpler metabolites (D'Souza *et al.* 2017).

CONCLUSIONS

In this study, a biocatalytic and recyclable GO-PVA composite membrane was obtained by immobilizing GO nanoparticles with PVA solution. After the addition of GO-PVA membrane, the degradation rate of CR was increased by up to four times, and the degradation efficiency was also increased from 42 to 95%. Moreover, GO-PVA membrane has good cyclability, and

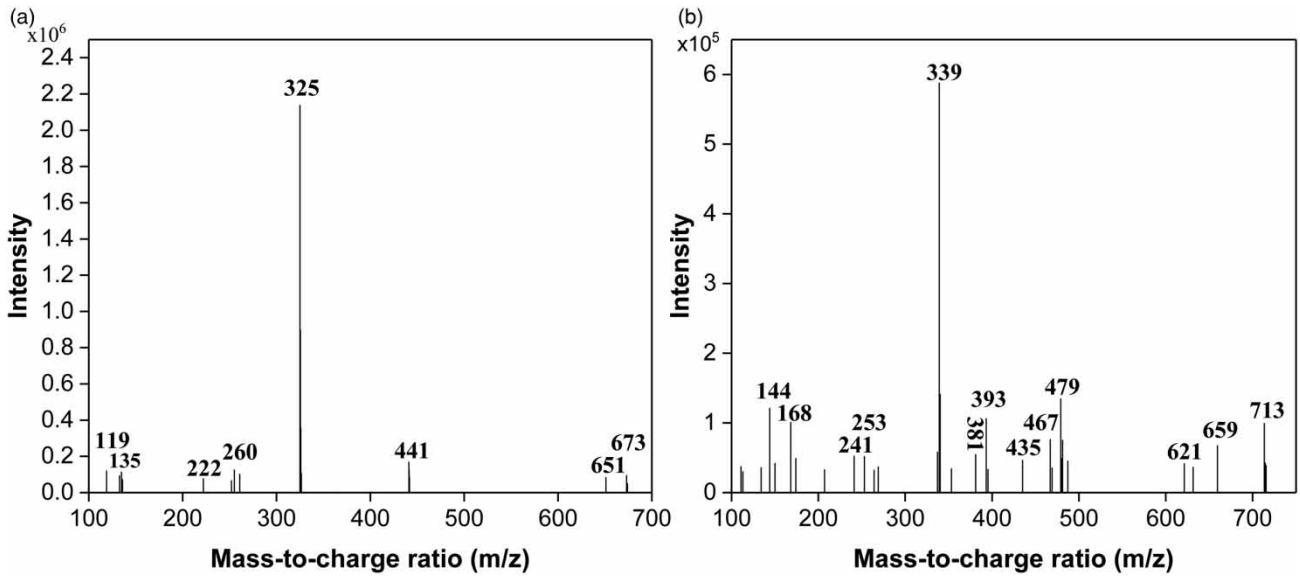

Figure 7 | ESI-MS spectrum of CR solution (a) before degradation (b) after 72 h degradation.

its biocatalyst performance does not decay after 10 cycle experiments. The degradation products of CR were confirmed by UV-vis and FTIR spectroscopy, and ESI-MS analysis, and the possible degradation mechanism of CR was proposed such that the CR gradually lost the -SO_3Na, -NH_2, and -N = N- groups and then was degraded as low-toxicity small-molecule aromatic compounds. The reason why GO-PVA membranes can accelerate the biodegradation of CR is that bacteria grow on the surface of GO and GO attracts CR molecules, which improve the electron transfer rate between bacteria and CR molecules. The results of this study are helpful to better understand the mechanism of *Shewanella* degradation of azo dyes and the application of bacteria in the bioremediation of dye wastewater.

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CONFLICTS OF INTEREST

There are no conflicts to declare.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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