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Enhanced production of microalgae-originated photosensitizer by integrating photosynthetic electrons extraction and antibiotic induction towards photocatalytic degradation of antibiotic: A novel complementary treatment process for antibiotic removal from effluent of conventional biological wastewater treatment

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ABSTRACT

Antibiotic residues in effluents from bio-treated wastewaters are mainly responsible for the spread of antibiotic resistance genes in the environment. Conventional physicochemical treatments are thought to be unsustainable due to high energy consumption, large consumption of chemicals and environmental unfriendly processing step. In this study, a novel approach by integrating photosynthetic electrons extraction from microalgae with antibiotic induction was used to enhance the production of microalgae-originated photosensitizer for photolytic removal of antibiotic residues in effluents from conventional bio-treated wastewaters. Results showed that the accumulation of photoactive substances in extracellular polymeric substance (EPS) of chlorella vulgaris was positively related to the amounts of photosynthetic electrons extracted by the electrode which is a potential-dependent process and can be further enhanced by tetracycline (TC) induction. The protein and humic acid which are considered two main photoactive substances in EPS produced at 0.6 V accumulated to a high level of 320 and 24 μg/cm^3 and were further increased to 380 and 48 μg/cm^3 when TC was added which were 4.7 and 6.4-folds higher than that produced at potential free in the absence of TC. The EPS produced at 0.6 and 0.8 V led to 1.34 and 1.53-fold acceleration in photosensitized degradation of TC compared to that of EPS free in secondary effluent of municipal wastewater treatment plant. The complex heterocyclic ring structure of TC was broken down into simple monocyclic aromatic compounds, indicating a marked reduction in biotoxicity and recalcitrance. The hydroxyl radical played a main role for the photolysis of TC followed by singlet oxygen. This technology provides a new alternative to conventional physicochemical treatment as complementary treatment processes for biological wastewater treatment in terms of antibiotics removal.

1. Introduction

Antibiotics, which are termed as emerging organic pollutants, have caused a serious hazard to the environment and human health in recent years due to their environmental persistence and spread of resistance genes (Singh et al., 2019). Antibiotics are widely present in a variety of wastewaters, such as municipal wastewater (Wang et al., 2020) and the effluent from livestock husbandry (Zhi et al., 2020) and the pharmaceutical industry (Hou et al., 2019). Among all commercially available antibiotics, tetracyclines (TCs) have been ranked second in terms of production and usage (Shi et al., 2013). However, due to their low bioavailability, 70%–90% of the TCs are excreted into the environment through urine and feces (Chen et al., 2016). TC has the highest frequency of detection in the effluents of municipal wastewater treatment plants, with residual concentrations ranging from 0.15 to 0.97 μg/L (Miao et al., 2004; Qiao et al., 2018) and in swine wastewater it

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reached a maximum of 300 mg/mL (Dong et al., 2020).

Biological methods are now the mainstream technologies for treatment of antibiotic-containing wastewaters because antibiotics are always coexist with nutrients which can be easily assimilated by microorganisms and are cost-effective and environmentally friendly treatments (Phoo et al., 2020). Although conventional biological wastewater treatment technologies supply a highly efficient removal of organic substance and N, P but show little degradation on antibiotics due to their strong antimicrobial activity and highly recalcitrant nature. The antibiotic residues in effluents from the bio-treated wastewaters are mainly responsible for the spread of antibiotic resistance genes in environment (Wang et al., 2020)).

Physicochemical methods have been demonstrated to be an effective complementary treatment processes for conventional biological wastewater treatment to eliminate antibiotics and avoid being discharged into recipient water bodies (Wang et al., 2020). Adsorption treatment, such as the use of activated carbon could achieve fast removal of antibiotic residues from water with a removal efficiency exceeding 90%, however, the adsorption only transfers, but, does not completely eliminate pollutants (Homem and Santos, 2011), and the recovery of adsorbent after desorption of pollutants is also a problem yet to be solved (Kamali et al., 2021). Advanced oxidation processes can generate reactive free radicals, such as hydroxyl radical (•OH) through Fenton reaction (Liu et al., 2018) or sulfate radical ($SO_4^{\bullet-}$) produced from peroxysulfate species (Zhou et al., 2019) with oxidative activity to destroy various hazardous organic contaminants. The main drawbacks of the conventional advanced oxidation process by adding directly the chemical agents are energy-intensive, heavy-consumption of chemicals and environmental unfriendly processing steps such as post-treatment. Photocatalysis technology is an attractive alternative to other advanced oxidation technologies, due to its green energy characteristics, mild reaction conditions and ability to produce rich free radical species. The artificial metal-based photocatalysts have been widely applied toward the removal of aqueous organic pollutants with high efficiency (Yousefi et al., 2016, 2019, 2021), but suffers from disadvantages such as high production cost, environmentally unfriendly synthesis steps and dissolution of potentially toxic elements. It is urgent to develop a cost-effective, environmentally friendly and highly efficient alternative/new photocatalysts to remove antibiotic residues from effluents of the bio-treated wastewaters before final discharge into environment.

Recently, microalgae-induced indirect photodegradation of antibiotics has aroused a wide attention (Wei et al., 2021). The mechanism involved in this reaction is that microalgae excrete extracellular organic matters which can work as photosensitizer to induce generation of active species for oxidative degradation of antibiotics. Significant efforts have been conducted to use the microalgae cell directly for antibiotics removal. However, there are several drawbacks and safety risks to this approach: (1) toxic effects of antibiotics and unfavorable growth conditions could inhibit photosynthetic metabolism of microalgae, leading to a low photosensitizer yield (Shang et al., 2015); (2) microalgae growth is susceptible to wastewater compositions and environmental conditions which make the regulation of the microalgae-based antibiotics treatment performance more complex; (3) antibiotic resistance genes may transfer and accumulate after the microalgal treatment of antibiotics (Wei et al., 2021). Extraction of extracellular photosensitizer from microalgae could be a good alternative to avoid the technical drawbacks mentioned above. The treatment of the residual antibiotics can be easily achieved by simple adding the microalgae-originated photosensitizer into wastewaters, and its treatment performance can be regulated by changing the doses of photosensitizer. What the most important is that this approach eliminates occurrence of antibiotic resistance genes which significantly reduces the environmental risk of the effluent from bio-treated wastewaters. The use of microalgae-originated photosensitizer and its enhancement production strategy for photocatalytic removal of antibiotic from wastewater are totally unexplored.

Photosynthetic metabolism largely affects the efficiency of pollutants removal by microalgae and the energy for photosynthetic metabolism is originated from photosynthetic electron transportation within the microalgal cell since it drives the formation of transmembrane proton gradient for ATP synthesis (Schuller et al., 2020). Previous studies suggested that the photosynthetic electrons produced by photosystem II are usually not fully released due to the kinetic limiting transfer step located downstream of photosystem II (Roach and Krieger-Liszkay, 2014). It is therefore reasonable to assume that extraction of photosynthetic electrons from the photosynthetic electron transport chain could be a potential approach to promote the photosynthetic metabolism of microalgae and thus stimulating the excretion of microalgae-originated photosensitizer. Addition of low-concentration target pollutant has also been proved effective in stimulating microbial metabolism. Hou et al. (2019) found that low concentration of toxic aniline could promote anaerobic biofilm development by stimulating the production of extracellular polymeric substances (EPS). The dose-dependent stimulation of bacterial biofilm formation by subinhibitory concentrations of antimicrobials has also been reported. And enhanced production of extracellular matrix components was proposed as one of the mechanisms (Ranieri et al., 2018). Although the photosynthetic metabolism of microalgae is distinctly different from the respiratory metabolism of bacteria, the low concentration of antibiotics could still be a potential approach for stimulating microalgae-originated photosensitizer synthesis, which has not yet been explored.

In present study, we make a first attempt to stimulate microalgae-originated photosensitizer production by integrating photosynthetic electrons extraction using poised solid matrix as extracellular photosynthetic electron receptor and antibiotic induction. The microalgae-originated photosensitizer was then extracted and directly applied to the photocatalytic removal of antibiotic in wastewater. *Chlorella vulgaris*, widely used for wastewater treatment, was used as biocatalyst for antibiotic degradation and TC was selected as a model antibiotic. Firstly, the effect of photosynthetic electron extraction rate controlled by applying different electrode potentials on the yield and the components of extracellular organic matters produced by C*hlorella vulgaris* were analyzed. Then, the extracellular organic matters were analyzed through three-dimensional fluorescence spectroscopy and confocal fluorescence microscopy to detect possible photosensitive substances and their distribution within the C*hlorella vulgaris* biofilm. Lastly, the EPS extracted from C*hlorella vulgaris* grown at different potentials were tested to determine its role in the photosensitive degradation of TC. The results of the present study propose a theory for the development of a clean, energy-saving and eco-friendly technical approach for enhancing removal of antibiotic, which has the potential to be applied to the advance treatment of bio-treated wastewaters effluent containing antibiotics residues.

2. Materials and methods

2.1. Chemicals

Tetracycline hydrochloride ($C_{22}H_{24}N_2O_8$·HCl, >95%) was purchased from Aladdin Industrial Corporation (Shanghai, China). All the other chemicals of analytical grade were purchased from commercial sources. Acetonitrile and the formic acid employed as the mobile phase of HPLC were of chromatographic grade (>99.9%) and obtained from Aladdin Industrial Corporation. The water used in the experiment was ultra-pure water from Unique-R20 (Xiamen, China).

2.2. Electrochemical device for microalgal photosynthetic electron extraction

The three electrode electrochemical devices contained a 100 mL glass bottle. Carbon felt (3 × 2 × 0.5 cm) and titanium wire (diameter = 0.05 cm) were employed as the working and counter electrodes. Prior to

usage, the carbon felts were immersed in acetone and sonicated for 10 min, and then soaked in hydrochloric acid solution (5 mol/L) for 1 min to remove impurities. The titanium wire was wound into a 12-turn spring (diameter = 1 cm, height = 3 cm). The two electrodes were placed in parallel in the center of the device with a distance of approximately 1.5 cm between them. The saturated calomel electrode (SCE, +0.242 V vs. standard hydrogen electrode (SHE), Leici, China) was then inserted between the two initial electrodes. The device was connected to a gas collector. All gaps in the device were sealed with epoxy resin. Fig. 1 presents a schematic diagram of the three electrode electrochemical devices.

Chlorella vulgaris isolated from the South China Basin was inoculated into the three-electrode electrochemical devices. The growth medium used for incubation contained (per liter):2 g $NaHCO_3$, 7.76 g $K_2HPO_4{\cdot}3H_2O$, 2.53 g KH_2PO_4, 0.31 g NH_4Cl, 0.13 g KCl, 0.01 g EDTA-Na_2, 0.06 g Citric acid, 0.06 g Ferric citrate, 19 mL of mineral solution and 1.9 mL vitamin solution (Sun et al., 2015). Following the sterilization of the reactors and cultivating mediums at high temperature, *Chlorella vulgaris* and the cultivating medium were added into the reactor in a sterile operation box. *Chlorella vulgaris* was acclimated by applying several electrode potentials to the working electrode via multi-channel potentiostat (CHI 1000C, Chenhua, China). The driving forces applied to extract the photosynthetic electron varied with the electrode potentials. When the photosynthetic current stabilized, the domestication was successful, and the domesticized *Chlorella vulgaris* were denoted as electroactive algae. The *Chlorella vulgaris* that had not undergone electrode potential domestication were denoted as non-electroactive algae. During the experiment, a cold white fluorescent lamp (3000 Lux) was used as light source to simulate the sunlight for photosynthesis, with the distance between the light source and working electrode set as 5 cm. The entire experiment was performed at a constant temperature of 28 ± 1 °C under light: dark = 12 h: 12 h. Unless otherwise specified, all electrode potentials are subsequently referred to as SCE.

2.3. Integrating photosynthetic electrons extraction and antibiotic induction for enhancing accumulation of photoactive substances in EPS

The potentials of working electrode were set to 0.4, 0.6 and 0.8 V by using a potentiostat in the presence of TC to investigate the contribution of the integrating photosynthetic electrons extraction and antibiotic induction to photoactive substances accumulation in EPS, with the EPSs extracted from *Chlorella vulgaris* grown at potential free and TC free as controls.

2.4. Photodegradation of TC by the Chlorella vulgaris-originated biophotosensitizers with different photoactive substance contents

In order to test the possibility of the *Chlorella vulgaris*-originated EPS to function as a biophotosensitizer for inducing photodegradation of TC and to determine the role of electrochemistry and antibiotic enhanced accumulation of the photoactive substances in EPS for enhancing the photodegradation of TC, the EPS extracted from *Chlorella vulgaris* grown under different external potentials was added to the secondary effluent from a local municipal wastewater treatment plant in Guangzhou city with additional supplement of 1 mg/L TC. The degradation dynamics of TC was monitored and the possible reactive free radicals responsible for the photodegradation of TC were identified.

2.5. Analytical methods

2.5.1. Electrochemistry analysis

The photosynthetic electron extraction from the *Chlorella vulgaris* was transformed to an electrical signal that was recorded every 1 min using the multi-channel potentiostat detailed in Section 2.3. Cyclic voltammograms (CV) and electrochemical impedance spectroscopy (EIS) were performed via a single-channel potentiostat (CHI 660, Chenhua, China) to test the electrochemical activity and photosynthetic electron transfer rate from the *Chlorella vulgaris* biofilm. The CV parameters were set with a scan rate of 5 mV/s from −0.5 to 0.5 V. EIS was measured over a frequency range of 0.1 MHz–5 mHz with a perturbation amplitude of 5 mV. The EIS data were fitted by using ZSimDemo.

2.5.2. TC degradation analysis

TC concentrations were detected by high performance liquid chromatography (HPLC, LC-16, Jiangsu, China). The TC mobiles contained HPLC grade acetonitrile and 0.1% formic acid (v/v = 20/80) at a flow rate of 1.0 mL min^{-1}. TC was detected at 280 nm and the analytes were separated in an C18 column (5 μm; 4.6 × 150 mm, phenomenex, CA, USA). All samples were filtered by a 0.22 μm water filter and stored in a refrigerator at 4 °C for testing.

The transformed TC products were identified by ultra-performance liquid chromatography Q-Exactive Orbitrap mass spectrometry (UPLC-Q-Orbitrap MS, Thermo Fisher Scientific, CA, USA). The specific test method of transformed TC products was shown in Method S1.

In order to further explain the contribution of microalgae-based

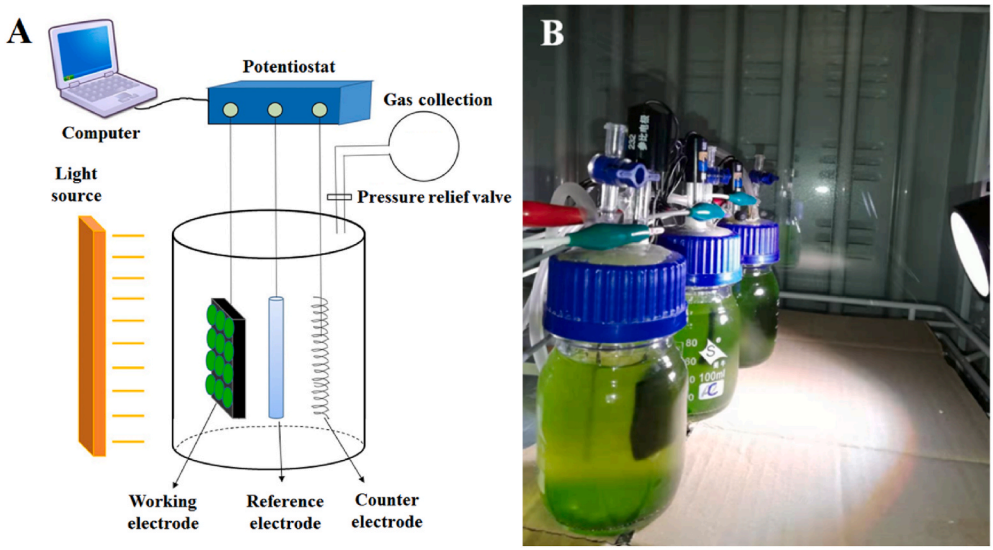

Fig. 1. The schematic diagram (A) and picture (B) of the electrochemical device used for extraction of photosynthetic electrons from *Chlorella vulgaris*.

process, electrochemical oxidation, direct photolysis and hydrolysis to TC removal in the three-electrode electrochemical system, the same time periods (0.4 V-24 h; 0.6 V-8 h; 0.8 V-6 h) were selected for analysis when the removal rate reaches 90% at each potential. The specific calculation formulas were shown in Method S2.

2.5.3. Reactive free radicals identification

Electron paramagnetic resonance (EPR, EMXPlus-10/12, Bruker, Germany) analysis were performed for identification of the possible reactive oxygen species (ROS), responsible for the TC photodegradation induced by EPS of *Chlorella vulgaris* grown under different external potentials.

2.5.4. EPS characterization

2.5.4.1. EPS extraction. The EPS from *Chlorella vulgaris* biofilms were extracted by following the method in the previous literature (Hou et al., 2020). In brief, the graphite felt electrodes were carefully sampled from each reactor. The electrodes were firstly resuspended in 4 mL of NaCl solution (0.9%). The suspension was further diluted with 4 mL of NaCl solution (0.9%) preheated to 70 °C to maintain a temperature of 35 °C in the final solution. The solution was then sonicated for 2 min for the separation of EPS. The electrode was removed from the solution, rinsed with an additional 2 mL of NaCl solution (0.9%) and discarded. The suspension was subsequently sheared by a vortex mixer for 2 min and centrifuged at 5000×*g* for 15 min. The supernatant was filtered through a 0.45 μm membrane filter. The supernatant organic matters were collected as the loosely bound EPS (LB-EPS).

The remaining algal cells in the centrifuge tube were again resuspended by 10 mL of 0.9% NaCl solution and heated at 50 °C for 30 min. The heated solution was then centrifuged at 5000 g for 20 min. The supernatant was filtered through a 0.45 μm membrane filter as the tightly bound EPS (TB-EPS) (Wang et al., 2016).

2.5.4.2. EPS components and distribution analysis. The protein in the collected EPS was detected via a BCA Protein concentration determination kit (Coolaber, SK1070). The Polysaccharides and humic acid were measured by the anthrone method and a modified Lowry method (Larsson, G. and M. Tornkvist, 1996). In order to analyze comprehensively the influence of extracting photosynthetic electrons from *Chlorella vulgaris*, the EPS component concentrations were designated as a function of the volume of graphite felt (μg/cm^3).

A high-resolution confocal laser microscope (LSM 800 with Airscan, Carl Zeiss, Germany) was used to observe the differences in the spatial distribution of proteins, α-polysaccharides and β-polysaccharides in the EPS from the *Chlorella vulgaris* biofilm. The specific dyeing of *Chlorella vulgaris* biofilm method was shown in Method S3. Moreover, fluorescence measurements were performed via a three-dimensional fluorescence spectrometer (F-4600, Hitachi, Japan) equipped with a 450 W xenon lamp as the excitation light source. The specific test method of fluorescence measurements was shown in Method S4.

3. Results and discussion

3.1. Potential-dependent photosynthetic electrons extraction from *Chlorella vulgaris*

In order to investigate the effect of electrode potentials on photosynthetic extraction from *Chlorella vulgaris*, the photosynthetic current intensities produced at different electrode potentials were recorded. As

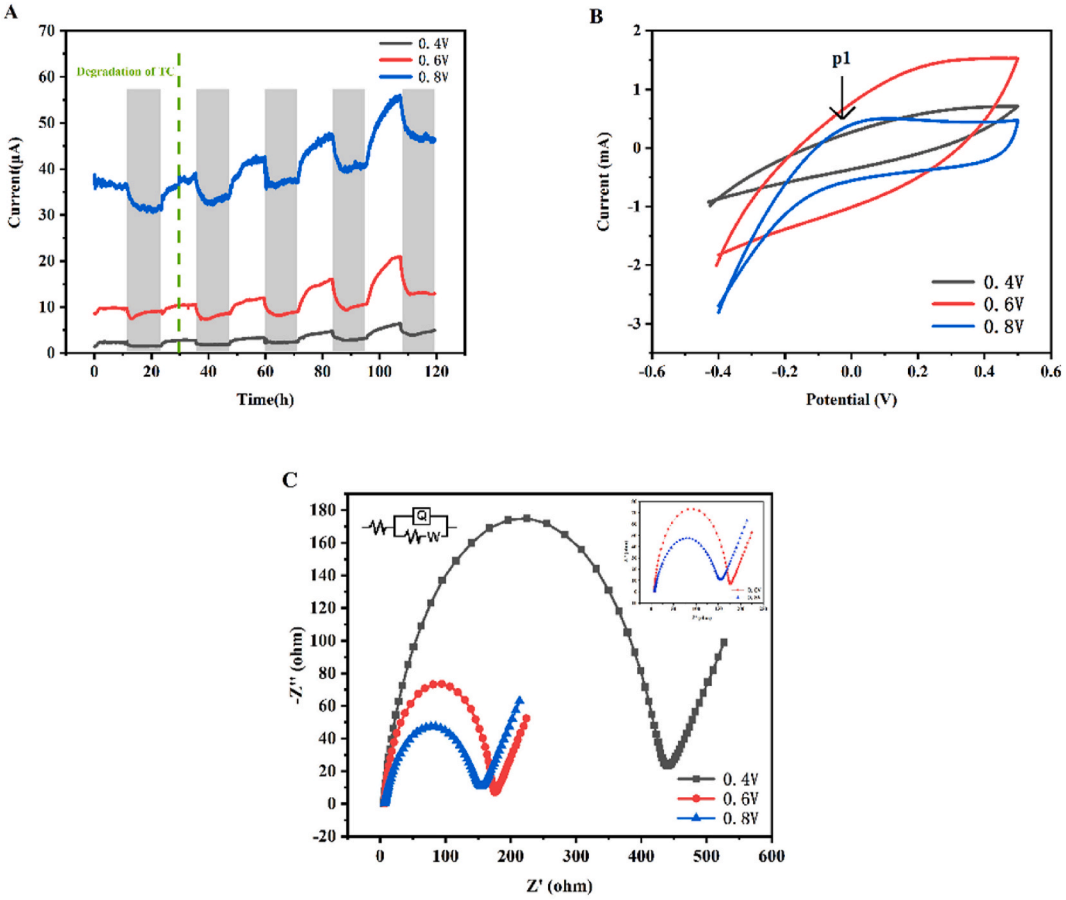

Fig. 2. The diagram of current-time (A), CV (B) and EIS (C) of *Chlorella vulgaris* biofilm grown at different electrode potentials in the presence of TC.

shown in Fig. 2 (A), the maximum photocurrent intensity of 6.43 μA, 20.93 μA and 56.03 μA at 0.4, 0.6 and 0.8 V were observed, indicating that the amounts of photosynthetic electrons extracted from *Chlorella vulgaris* were positively correlated to the electrode potentials. The larger photosynthetic current intensity at higher electrode potentials might be ascribed to stronger biofilm adhesion or a more efficient extracellular electron transfer path (Zhu et al., 2012). It was worth noting that e photosynthetic current intensity was relatively low during TC degradation, which might have been the result of the inhibition of the photosynthetic activity of *Chlorella vulgaris* due to the biotoxicity of TC (Zhao et al., 2020). The photosynthesis is considered as one of the competitive strategies to take benefits from the solar energy. However, it uses only a very small fraction from the solar energy (Blankenship et al., 2011) due to the fact that the photosynthetic electrons production by photosystems II was largely limited by the step located downstream of photosystem II and the photosystem II did not reach its maximal turnover rate. The highly efficient photosynthetic electrons extraction by using a poised electrode in this study demonstrated a great potential in utilizing the remarkable characteristics of photosystem II of microalgae to generate sustainable bioelectrical power during wastewater treatment which provide a convenient and effective approach to utilize the solar energy and resourceful treatment of wastewater.

To explore the mechanisms of photosynthetic electrons extraction from *Chlorella vulgaris* by the poised electrodes, CV scans of the *Chlorella vulgaris* biofilm under non-turnover conditions were conducted. As shown in Fig. 2(B), no obvious redox peaks were observed for the *Chlorella vulgaris* biofilm grown at 0.4 V and 0.6 V, while the extracellular photosynthetic electron transfer ability was improved for the latter compared with the former. However, the CV current response area at 0.8 V was lower than that of 0.6 V. This might be attributed to the damage of *Chlorella vulgaris* protein at high potential due to its negative impact on the microbial activity (TerAvest and Angenen, 2014) and the fact that electrons are more likely to be transferred to media with low activation energy (Call et al., 2017). Moreover, an oxidation peak (p1) was clearly observed at a peak potential of −0.05 V for the biofilm grown at 0.8 V, which could be attributed to the cytochrome (c-type) correlation (Srikanth et al., 2008). Photosynthetic algae can transfer metabolic electrons to extracellular electron receptors through the c-type chain on the cell membrane (Rosenbaum et al., 2011). The photosynthetic electron transfer pathway of *Chlorella vulgaris* grown at 0.8 V might be different from that grown at 0.4 and 0.6 V, resulting in higher photosynthetic electron transfer rate. This suggests that the strong oxidation potential (0.8 V) activated *Chlorella vulgaris* c-type to a greater extent than that at low potentials (0.4 V and 0.6 V).

Fig. 2(C) presents the EIS diagram of the *Chlorella vulgaris* biofilm under non-turnover conditions. The solution resistance (Rs), charge transfer internal resistance (Rct) and Warburg diffusion components (WDE) of the *Chlorella vulgaris* biofilm grown at different electrode potentials were obtained by fitting the Nyquist diagram to the corresponding equivalent circuit diagram (Fig. 2(C), inset) and the fitted resistance parameters were listed in Table S2 in the supplementary material. The Rct values for the biofilm grown at 0.4 V, 0.6 V and 0.8 V were 423 Ω, 165.1 Ω and 143.9 Ω. This indicates that the high electrode potential reduced the resistance of extracellular photosynthetic electron transfer, which contradicts the results of the photosynthetic bacteria (Sun et al., 2020). At 0.8 V, the appearance of the extracellular photosynthetic electron transport mediator (c-type) represented by the p1 peak in the CV test could enhance the competitiveness of the electrode to serve as an electron acceptor, resulting in the largest photosynthetic current intensity.

3.2. Effect of photosynthetic electrons extraction on TC removal by using microalgal cell directly

In order to investigate the effect of photosynthetic electrons extraction on TC removal by microalgal cell, the variation of TC concentration with time under different conditions were monitored. As shown in Fig. 3 (A), 93% TC was removed within 10 h by *Chlorella vulgaris* grown at 0.8 V while the removal efficiency was only 71% for *Chlorella vulgaris* grown at potential free. The degradation of TC exhibited a good fit with the first-order kinetic model, and the degradation rate constant at 0.4 V (0.11708 h^{-1}), 0.6 V (0.17696 h^{-1}) and 0.8 V (0.28747 h^{-1}) were 1.28, 1.93 and 3.14-folds higher than that at potential free (0.09143 h^{-1}) (Fig. 3(B)). Fig. 3(A) and (B) also indicated that single electrochemical oxidation contributed much less to TC degradation and the most of TC was removed by algae-based process.

To further explore the contribution of algae-based process, electrochemical oxidation, direct photolysis and hydrolysis to TC removal, the contribution ratio of each approach was calculated according to eqs. (1), (2), (3) and (4) in Method S1. As shown in Fig. 3(C), the contributions of algae-based process for TC removal at 0.4 V, 0.6 V and 0.8 V were 48%, 56% and 56%, indicating that the algae-based process was mainly responsible for TC removal during the photosynthetic electrons extraction. The higher electrode potentials (0.6 V and 0.8 V) are more prone to increase the removal ratio of TC by the algae-based process than that at low electrode potential (0.4 V), which might be attributed to the higher photosynthetic metabolic rate of alga stimulated by highly efficient photosynthetic electrons extraction at high potentials.

3.3. Effect of integrating photosynthetic electrons extraction and antibiotic induction on photoactive substances accumulation in EPS of microalgae

3.3.1. Possible photoactive substances analysis in EPS

As previously reported, the EPS could work as photosensitizer which plays a dominant role in photocatalytic degradation of antibiotic (Wei et al., 2021). To confirm the influence of photosynthetic electrons extraction and TC addition on photoactive substances content in EPS of *Chlorella vulgaris*, the protein, polysaccharide and humic acid content under different conditions were measured. The EPS were divided into loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS) based on their composition structure. As shown in Fig. 4, photosynthetic electrons extraction promoted significantly the protein, polysaccharide and humic acid accumulation in EPS of *Chlorella vulgaris*. The contents of protein, polysaccharide and humic acid in total EPS produced at 0.6 V accumulated to a high level of 320, 27 and 24 μg/cm^3 which were 3.76, 2.25 and 2.1-folds higher that produced at potential free, respectively. The contents of them increased further to 380, 28 and 48 μg/cm^3 when TC was added, indicating the synergistic effect of photosynthetic electrons extraction and TC induction on enhancement of each component synthesis in EPS. It was found that the potential regulating photosynthetic electrons extraction rates had a great influence on each component content in EPS. The increases in protein and polysaccharide content were not proportional to the rise in potentials and the highest protein content in total EPS was achieved at 0.6 V while the other was achieved at 0.8 V in the presence of TC. In comparison, the rise in potentials led to a continuous increase in humic acid content in total EPS. The content of humic acid in total EPS produced at 0.4, 0.6 and 0.8 V were 2.5, 4.6 and 6.5-folds higher than that of EPS produced at potential free in the absence of TC. The variation of each component in LB-EPS and TB-EPS exhibited a trend similar to that of changes in total EPS under different production conditions. It is worth noting that the humic acid content increased more notable through photosynthetic electrons extraction and TC induction and was mainly accumulation in inner-layer TB-EPS. It is well known that humic substances can act as photosensitizer and greatly affect the photodegradation of toxic organic micro-pollutants (Chen et al., 2017). The proteins involved in photosynthesis might be also seen as photosensitizer, but there lacks enough evidence to support polysaccharide's functioning as photosensitizer at present (Wei et al., 2021). High accumulation of photosynthetic protein and humic acid in EPS produced with applied potentials indicating that photosynthetic electrons extraction coupling with TC induction was an effective approach to

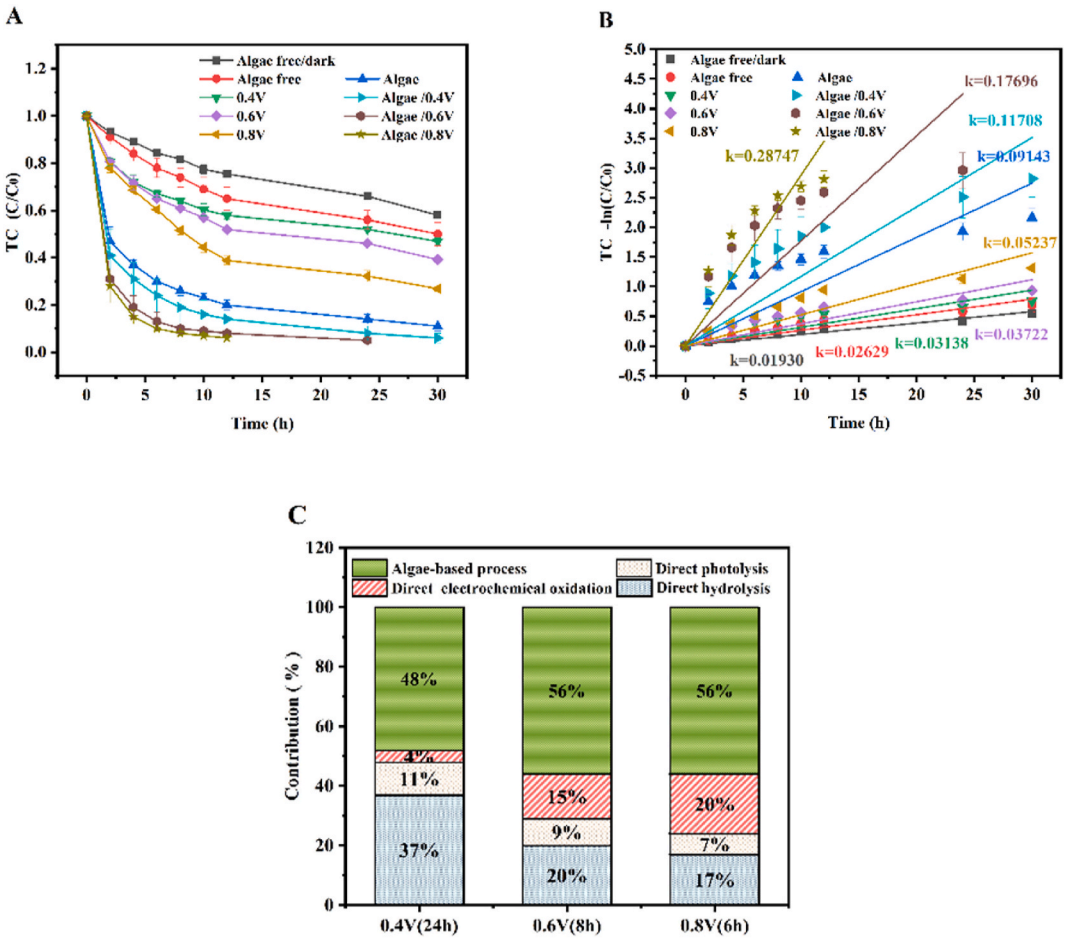

Fig. 3. Removal efficiency (A) and kinetics (B) of TC by *Chlorella vulgaris* grown under different conditions, and the contribution ratio of different approaches to TC removal at different applied potentials (C).

directionally accumulate microalgae-originated photosensitizer in EPS.

Three-dimensional fluorescence spectral measurements have been proven to be effective in the characterization of fluorescent substances in EPS. We employed this method to further analyze the effects of photosynthetic electrons extraction and TC induction on photoactive substance accumulation in EPS. Table S3 (in the supplementary material) and Fig. 5 demonstrated the specific parameters (position, fluorescence type, and fluorescence intensity) used for the measurements. Although both peaks represent protein-like substances, peak A (Ex/Em 275/320–350) indicates a protein-like substance containing tryptophan, while peak B (Ex/Em 235/335–345) belongs to aromatic protein (Pan et al., 2020). The appearance of peak C (Ex/Em 330/415–430) may be attributed to the death of algae cells (Qu et al., 2012). The appearance of peak D (Ex/Em 240/410–450) and peak E (Ex/Em 245/445) were associated with fulvic acid-like substances (Hou et al.). Peak F (Ex/Em 265/450) represents humic acid-like substances (Yan et al., 2019). The fluorescent intensity of Peak D and F in EPS produced at 0.6 and 0.8 V increased by 297% and 189% compared with those in EPS produced at potential free, suggesting a high accumulation of humic and fulvic acid-like substances

The fluorescence intensities of Peaks A, D and F in the EPS produced at 0.4 V (Fig. 5 (C)) were weaker than those of EPS produced at 0.6 V and 0.8 V, indicating a weak stimulating effect on tryptophan protein, humic and fulvic acid-like substances accumulation at that potential.

A comparison of the fluorescent intensity of EPSs produced at 0.6 V in the presence and absence of TC revealed that TC addition increased the fluorescence intensity of peak D significantly (Fig. 5 (B) and (D)), indicating that TC addition promoted the fulvic acid-like substances accumulation in EPS, which is consistent with the results of Ma(Ma et al., 2020). These results suggested that TC was able to promote microalgae to extracellular synthesis of humic and fulvic acid-like substances which can be extracted as biophotosensitizer. The TC might act as signal molecule to stimulate production of fulvic acid-like photoactive substances in EPS (Ranieri et al., 2018).

As previously reported, many substances produced by algae can act as photosensitizers such as chlorophyll, enzymes and extracellular organic matters (Pivokonsky et al., 2014). Among these photosensitizers, the chlorophyll and intracellular enzymes could contribute little to the photocatalytic removal of antibiotic since the reactive oxidative species generated by them could not pass through the cell membrane to contact with antibiotics. Extracellular enzymes could also be a potential photosensitizer but their roles in antibiotic photodegradation are not currently evident (Tian et al., 2019). The humic and fulvic acid-like substances whose relative proportion increased significantly in the EPSs of algae with high photosynthetic electrons extraction efficiency (at 0.6 and 0.8 V) obviously own high capability for inducing photocatalytic degradation of antibiotic.

3.3.2. Spatial distribution of EPS in Chlorella vulgaris biofilm

In order to clarify the technical advantage of photosynthetic electrons extraction for recovery of microalgal biomass for photosensitizer production by using the photosensitizer extracted from microalgae over the use of the microalgal cell which containing photosensitizer directly for antibiotic removal, the microalgal biomass, and spatial distribution and visualization of EPS within the *Chlorella vulgaris* biofilm were analyzed via a high-resolution confocal laser microscope. The specific

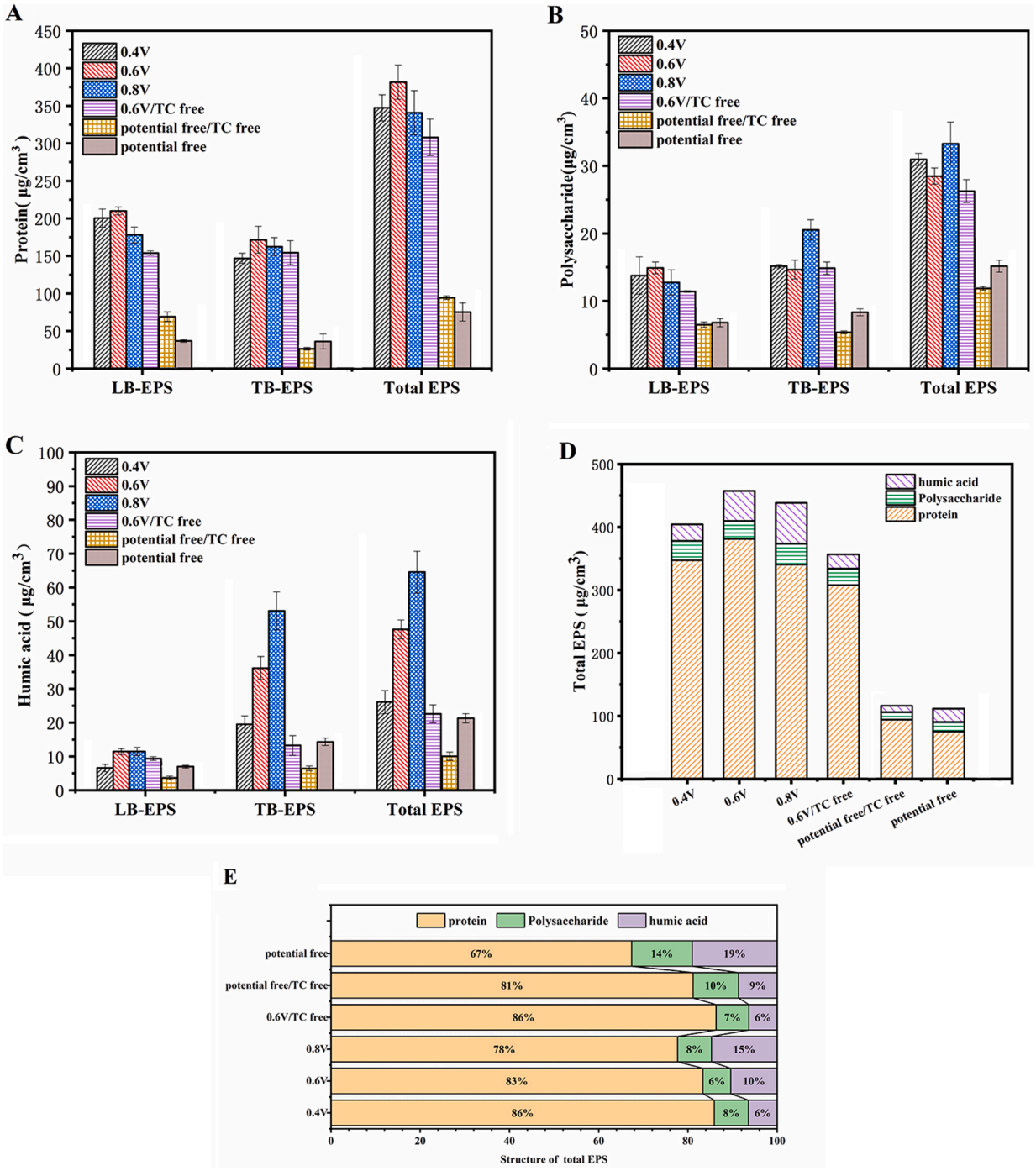

Fig. 4. The contents of protein (A) polysaccharide (B) and humic acid (C) in LB-EPS, TB-EPS and total EPS (D), and their proportions in total EPS under different operational conditions.

and three-dimensional views were presented in Fig. 6, where protein, α-polysaccharide and β-polysaccharide were represented in green, red and blue. The spatial distribution ratio of each component of EPS within the *Chlorella vulgaris* biofilm was calculated from the fluorescence intensity of each biofilm (Tables S1 and S4). As shown in Fig. 6, the *Chlorella vulgaris* biofilm thicknesses were 840 μm at 0.4 V, 920 μm at 0.6 V and 840 μm at 0.8 V, while the potential free case exhibited the thinnest biofilm of 700 μm. These observed results demonstrated that photosynthetic electrons extraction enhanced significantly *Chlorella vulgaris* biofilm formation on surface of poised solid matrix which was a great convenience to recover microalgal biomass for microalgae-originated photosensitizer extraction, and 0.6 V was the most beneficial potential to recover *Chlorella vulgaris* biomass. The proteins, α-polysaccharides and β-polysaccharides within the biofilm were interlaced. The β-polysaccharides were considered to be the most important component of particle stability (Adav et al., 2008). The β-polysaccharides were evenly distributed on the surface of the *Chlorella vulgaris* biofilm grown at 0.4, 0.6 and 0.8 V, forming a continuous structure to support the biofilm. However, the β-polysaccharides distribution in biofilm grown at potential free was uneven, leading to a weak biofilm stability. The potential also affected the spatial distribution of each functional substances within the biofilm. As shown in Table S4 in the supplementary material, the proportion of proteins, α-polysaccharides and β-polysaccharides in the inner layer of the biofilm gradually

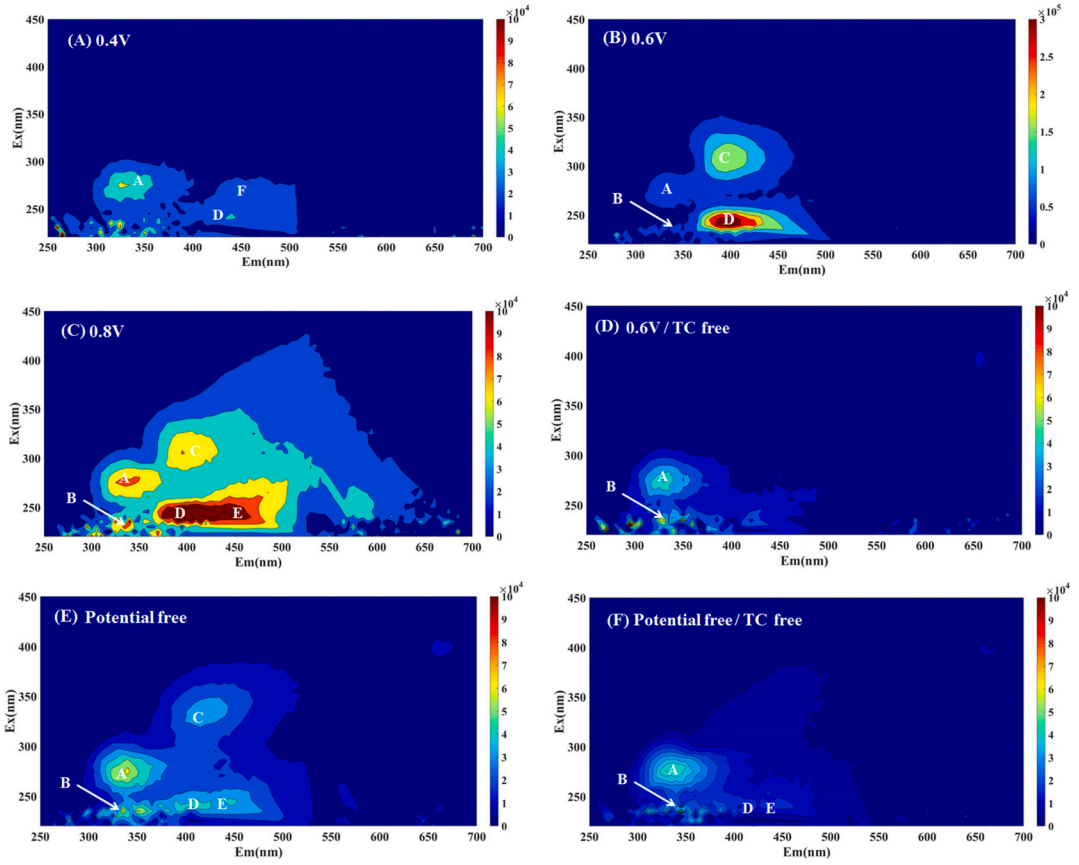

Fig. 5. Three dimensional fluorescence spectrum of EPS extracted from *Chlorella vulgaris* grown at different applied potentials in the presence or absence of TC.

increased with the applied potentials, while the middle layer exhibited the opposite trend (Table S4 in the supplementary material). It is important to note that large proportion of functional substance were located in middle and inner layer of the biofilm which prevents the interaction between photoactive substances and antibiotic, leading to a low photosensitive degradation efficiency. Whereas the use of photosensitizer extracted from microalgae could overcome this drawback.

3.4. Performance of photosensitizer extracted from Chlorella vulgaris for inducing photocatalytic degradation of TC

In order to determine the capability of photosensitizer extracted from *Chlorella vulgaris* for inducing indirect photocatalytic degradation of TC, the photocatalytic degradation rate of TC was compared in the presence and absence of photosensitizer, and under light and dark conditions. As is shown in Fig. 7, the photocatalytic degradation rate of TC by the EPS produced at 0.6 and 0.8 V were 1.34 and 1.53-folds higher than that produced at EPS free indicating that the EPS produced with the two applied potentials could significantly accelerate the removal of TC though inducing indirect photocatalytic degradation process. The degradation rate of TC was much lower under dark condition than that under light condition, indicating that EPS were not activated for photosensitive degradation of TC, and even exhibited a slight inhibitory effect on TC hydrolysis. It is noteworthy that the EPS produced at 0.4 V did not result in notable enhancement in inducing indirect photocatalytic degradation of TC, most likely due to the low levels of accumulation of humic and fulvic acid-like substances (Figs. 5 and 6). The EPS-induced photosensitive degradation of TC was a function of the type of free radicals or triplet excited-state extracellular organic matter (3EPS*) produced after the absorption of the excitation via the light photosensitizer (Wei et al., 2021). Photosynthetic electrons extraction at 0.6 and 0.8 V stimulated *Chlorella vulgaris* to secrete more humic and fulvic acid-like photosensitive substances, promoting the indirect photocatalytic degradation of TC. The above results indicated that the microalgae-originated photosensitizer could be applied directly for photocatalytic removal of antibiotic in wastewater with a high efficiency and at a very low adding concentration. This technology also possesses other merits of cost-effective, simple operation and environmental friendliness and the most important one is that the spread of antibiotic resistant genes was avoided, reducing significantly environmental risk of bio-treated wastewater. To further identify the active oxidizing specie types responsible for the TC photodegradation induced by EPS of *Chlorella vulgaris*, the EPR was conducted. As shown in Fig. S3, EPR detected high signal intensity of •OH followed by $^1O_2$. This result confirmed that there were •OH and $^1O_2$ produced in this process and •OH played a main role while $^1O_2$ played a minor role in TC photodegradation.

Positive and negative ion modes of LC-MS were employed to detect possible degradation products of TC. Fig. S1, Fig. S2 and Table S5 in the supplementary material present the corresponding LC-MS identification of the transformation products. Based on the transformation products detected by LC-MS, we proposed three possible degradation pathways of TC (Fig. 4). To obtain TP1 (m/z = 413), TC was dehydrated and demethylated. As C1–C2 formed a stable second aromatic ring, the dehydration site was most likely to occur in C3 (Liu et al., 2016). The majority of the demethylation products were produced in the photolysis process. TP2 (m/z = 406) was produced by protonation and the removal of the nitrogen-dimethyl bond with relatively low energy. Both TP3 (m/z = 301) and TP4 (m/z = 309) were formed by removing the nitrogen-dimethyl bond and hydroxyl from TP1. TP4 and TP5 (m/z = 391) also separated the amide group, while TP3 and TP6 (m/z = 321) were both produced by cleaving carbon atom ring A and opening the C4–C5 double bond and keeping the hydroxyl group. TP3 was also discovered in the research of Pan on the photocatalysis of TC (Pan et al.,

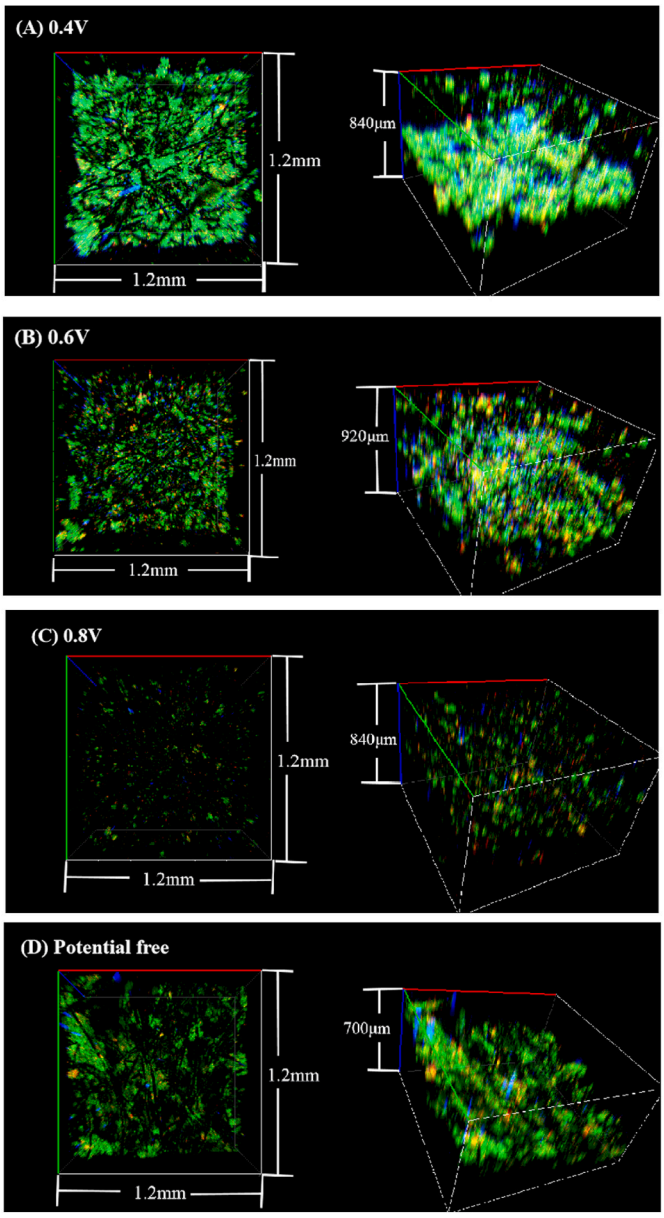

Fig. 6. Spatial distribution of EPS extracted from *Chlorella vulgaris* grown at different electrode potentials in the presence of TC.

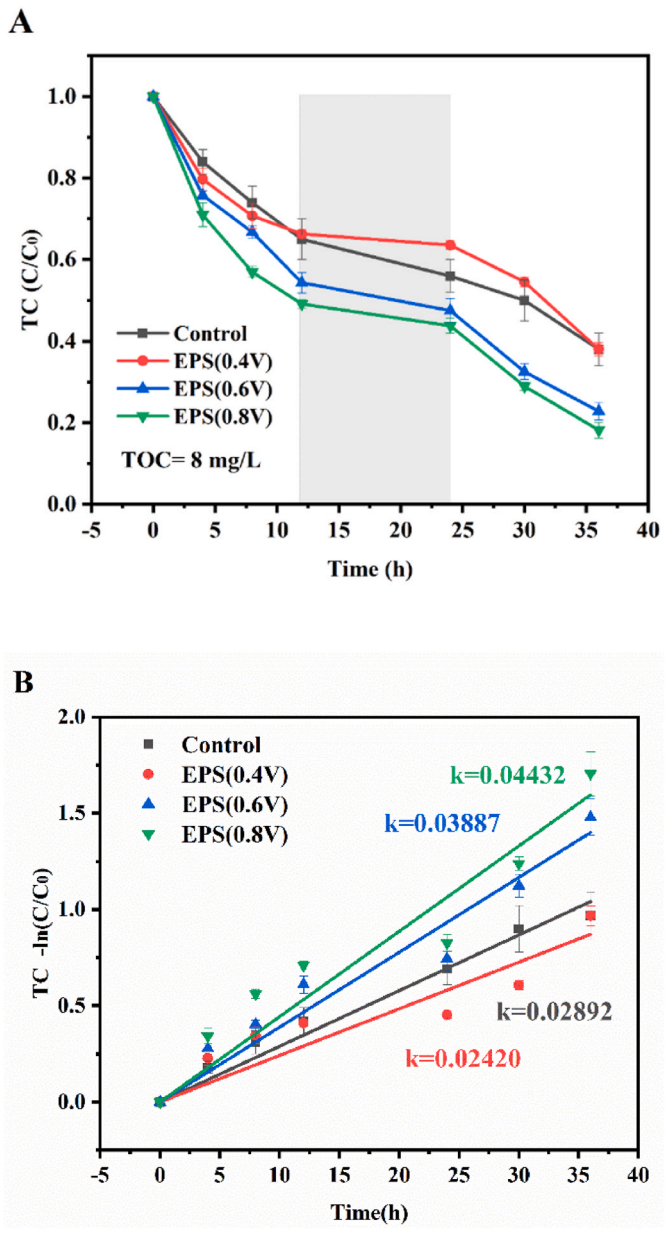

Fig. 7. Photosensitive degradation (A) and dynamics simulation of photosensitive degradation of (B) TC induced by EPS extracted from *Chlorella vulgaris* grown at different potentials in the presence of TC.

2020). The degradation pathways of these transformation products include terminal oxidation, dealkylation, dehydroxylation, deamination and ring opening (Li et al., 2020). With further degradation of the photo/biological reaction, macromolecular substances were successfully converted into small molecules with m/z of 215, 141 and 98, and subsequently into CO_2 and H_2O. Based on these results, complex heterocyclic ring structure of TC was broken down into simple monocyclic aromatic compounds and the biotoxicity of TC was largely reduced (Fig. 8). Microalgae-originated photosensitizer-induced indirect photolysis of TC provided an important approach to degrade TC in wastewater.

3.5. The possible mechanisms of the enhanced microalgae-originated photosensitizer production and its induced photolysis of antibiotic

Based on the above analysis, a mechanism of the enhanced production of microalgae-originated photosensitizer by combined electrical stimulation and antibiotic induction and the microalgae-originated photosensitizer induced photolysis of antibiotic is proposed (Fig. 9): the poised electrode can serve as extracellular electron acceptor for extracting photosynthetic electrons from the photosynthetic electron transfer chain within the microalgal cell, and the photosynthetic electrons extraction efficiency which directly affect the synthesis and composition of EPS can be controlled by adjusting electrode potential, and thus the photoactive substances were accumulated in EPS as a result of application of specific electrode potential (Hou et al., 2020). The stimulating effect for the accumulation of photoactive substances within EPS by poised electrode can be further enhanced by adding subinhibitory concentrations of antibiotic, attributing to the upregulation of genes related to EPS synthesis with antibiotic served as signal molecules (Ranieri et al., 2018).

3.6. Implication

It is well documented that algae show great potential for removing

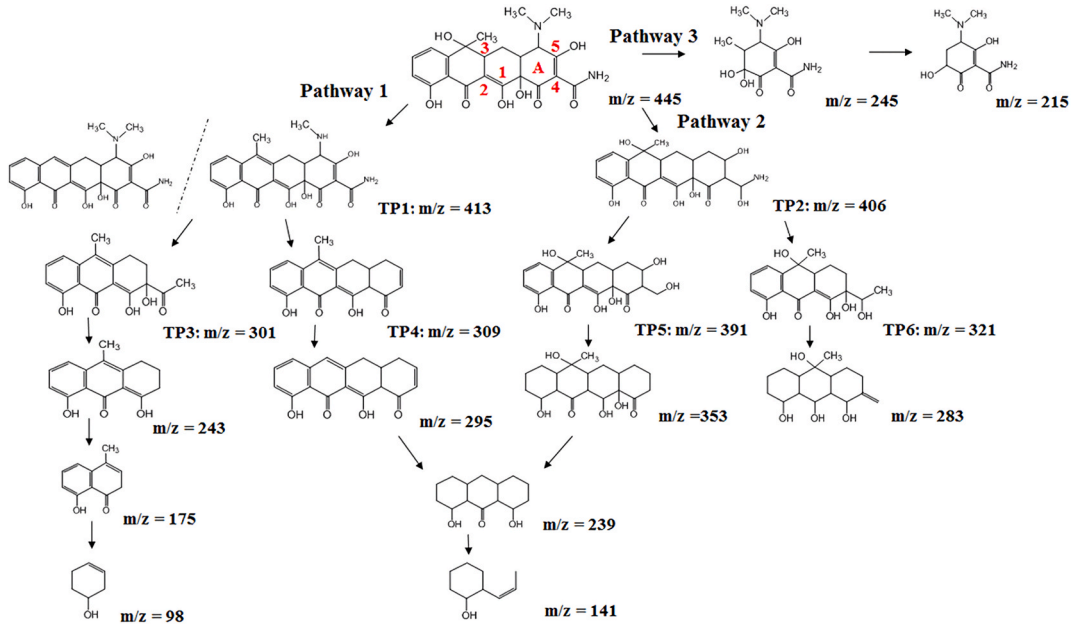

Fig. 8. Possible degradation pathways of TC in the presence of microalgae-originated photosensitizer.

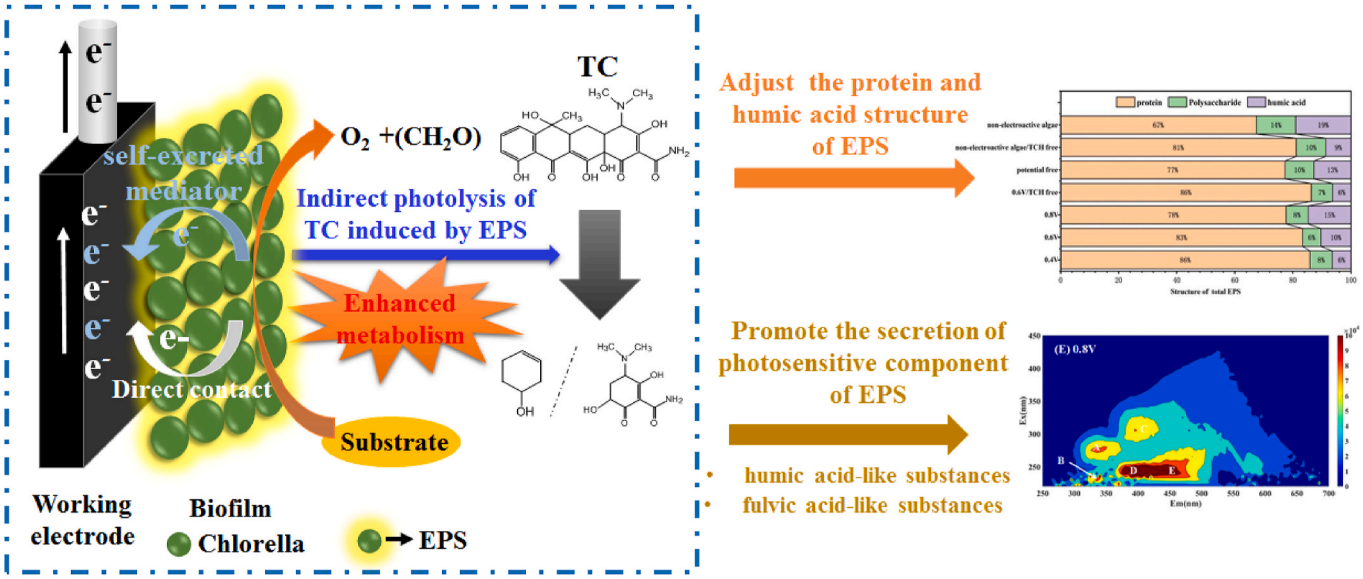

Fig. 9. Schematic mechanism of the enhanced production of microalgae-originated photosensitizer by combined electrical stimulation and antibiotic induction and the microalgae-originated photosensitizer induced photolysis of TC.

pharmaceutical contaminants. However, after the treatment of residual antibiotics by algae, antibiotic resistance genes may transfer and accumulate (Wei et al., 2021). In this study, the fast photolysis of TC in the presence of photoactive substances extracted from *Chlorella vulgaris* provided a novel technical approach to avoid such negative effects. Furthermore, the yield of microalgae-originated photosensitizer can be largely increased by electrical stimulation and antibiotic induction which is easy to operate. Moreover, a major advantage of using the microalgae-originated photosensitizer for aqueous antibiotic removal is that it eliminates the need for a noble or toxic metal and avoids large consumption of chemicals, thus providing an economical and environment-friendly alternative to traditional metal-based photocatalyst.

4. Conclusions

Extraction of photosynthetic electrons from microalgae coupling with antibiotic induction demonstrates an efficient and easily-manipulated technical approach for promoting microalgae-originated photosensitizer production and the photosensitizer extracted from microalgae exhibited extraordinary photosensitive activity for photocatalytic degradation of antibiotic. The accumulation of humic and fulvic acid-like photoactive substance through photosynthetic electron extraction was found to be a potential-dependent process and the 0.6 V was found to be the optimal potential. The combination of photosynthetic electron extraction with TC induction showed a synergistic effect on enhancement of photoactive substance accumulation in *Chlorella vulgaris*. The use of photosensitizer extracted from microalgae owns a great technical advantage over using microalgal cell directly for

antibiotic removal from wastewater in terms of photosensitized degradation efficiency. The technology explored in this study can be also considered a good alternative to physicochemical treatment to serve as a sustainable complementary treatment process for conventional wastewater treatment plants to eliminate the environmental risk from the discharge of antibiotic residues-containing effluent.

Credit author statement

Xiaoyan Bai: Conceptualization, Methodology, Formal analysis, Writing-review & editing. **Wanyi Liang:** Experiment, Data Curation, Software, Writing-original draft. **Jian Sun:** Project administration, Resources, Supervision, Writing-review & editing, Funding acquisition. **Chengxin Zhao:** Writing-review & editing. **Peng Wang:** Resources. **Yaping Zhang:** Resources, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2022.114527.

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