Biochar enhanced the performance of microalgae/bacteria consortium for insecticides removal from synthetic wastewater

Amin Mojiri[1*], John L. Zhou[2], Mansoureh Nazari V[3], Shahabaldin Rezania[4], Hossein Farraji[5], Mohammadtaghi Vakili[6]

[1]Department of Civil and Environmental Engineering, Graduate School of Advanced Science and Engineering, Hiroshima University, 1-4-1 Kagamiyama, Higashihiroshima 739-8527, Japan

[2]Centre for Green Technology, School of Civil and Environmental Engineering, University of Technology Sydney, 15 Broadway, NSW 2007, Australia

[3]School of Pharmacy, University of 17 August 1945, 14350 Jakarta, Indonesia

[4]Department of Environment and Energy, Sejong University, Seoul, 05006, South Korea

[5]School of Physical and Chemical Sciences, University of Canterbury, Christchurch, New Zealand

[6]Green intelligence Environmental School, Yangtze Normal University, Chongqing 408100, China

*Corresponding Author: Email: amin.mojiri@gmail.com OR mojiri@hiroshima-u.ac.jp

Abstract: The presence of pesticides in aquatic environments has threatened marine food resources, aquaculture, fisheries and human health; therefore, two most used insecticides were removed during this study. Two photobioreactors, including biochar and *Chlorella vulgaris*/activated sludge (reactor 1), and *Chlorella vulgaris*/activated sludge (reactor 2) were run to remove chlorpyrifos (CPF) and cypermethrin (CYP). *Proteobacteria*, *Bacteroidetes* and *Chloroflexi* were the dominant phyla of activated sludge. The optimization performance of both reactors was conducted by response surface methods. The performance of first photobioreactor was better than that in the second reactor, achieving abatement of 88.80% CPF and 93.12% CYP, at 69.7 h contact time and 0.32 mg/L initial concentration. The toxicity of CPF and CYP to *Chlorella vulgaris* was monitored under 0-4 mg/L of insecticide concentrations and 0-72 h contact time. The minimum chlorophyll content (2 mg/L) and protein (16.7%), and maximum growth inhibition (89.7%) were recorded at 4 mg/L insecticides concentration and 72 h contact time. Moreover, molecular docking simulation for catalytic enzyme degradation of *Proteobacteria*, *Bacteroidetes* and microalgae was carried out using individual hydrolase enzymes: carboxypeptidase in microalgae, isochorismatase hydrolase in *Proteobacteria* and alpha-L-arabinofuranosidase in *Bacteroidetes*. Ligand-binding energy, affinity and dimensions of ligands-binding sites in the enzyme cavity were calculated in each case. Hydrolase is an enzyme group that offers a promising practical application for the degradation of CYP and CPF due to its cavity features. This analysis demonstrated the mode of interaction of ligands with hydrolase enzymes in different species.

Keywords: Biochar; *Chlorella vulgaris*; Chlorpyrifos; Cypermethrin; Hybrid process

1. Introduction

Water is a vital natural resource that is essential for human sustainable development (Ma et al., 2020). With industrialization, urbanization and rapid human population growth, water contamination has become increasingly severe, hence affecting freshwater resources and becoming one of the main challenges of the 21st century (Zhang et al., 2021). Amongst the common contaminants, pesticides are of the main concern around the world as they are widely used, relatively stable and non-biodegradable, bioaccumulative, and highly toxic for the ecosystems and human health (Kamboh et al., 2021).

Pesticides have been employed to control pests and weed to improve the yield of the crops (Kodali et al., 2021). However, only around 1% of the applied pesticides reached the target organisms with the remaining 99% in soils and waterways (Ali et al., 2019). The contamination of aquatic environments by pesticides residues has threatened marine food resources, aquaculture and fisheries (Bilal et al., 2019, Morsi et al., 2020). Pesticides are categorized in three major groups as fungicides, herbicides, and insecticides. Insecticides are generally employed to control insects, but their environmental safety has become a serious concern. In 2016, 17% of the total applied pesticides (4.1 million tonnes) were insecticides (Mojiri et al., 2020).

Of wide concern are two common insecticides, chlorpyrifos (CPF) and cypermethrin. CPF (O,O-diethyl O-3,5,6-trichloropyridin-2-yl phosphorothioate) is an organophosphate insecticide, which has been remarked as the hazardous substance because of its residual presence and toxicity. Due to the large application of this insecticide, CPF is often reported in water sources and endangers the survival of animals, aquatic organisms and even humans (Hamadeen et al., 2021). Furthermore, the half-life of CPF in water sources is up to 77 days (Sheikhi et al., 2021). Tulun et al. (2021) expressed that CPF could cause the neurotoxicity in children. In addition, exposure to CP even at low concentrations has been reported to result in health problems such as lung cancers in humans. Pyrethroid insecticides are one of the most

frequently applied insecticides in agriculture due to their broad-spectrum insecticidal capability and high effectiveness. One of the most used pyrethroid insecticides is cypermethrin (CYP) (Zhang et al., 2021). CYP can cross the blood-brain barrier (BBB) to cause neurotoxicity in the nervous system (Ali et al., 2020). Moreover, the half-life of CYP in water sources is 8 to 16 days (Laskowski, 2002).

Different methods (e.g. physicochemical and biological methods) have been employed to remove pesticides from wastewater. Physicochemical techniques such as advanced oxidation process (AOP) are effective for removal of organic contaminants (such as pesticides) (Ding et al., 2020, Zhao et al., 2021), but they have some disadvantages, such as high energy and operational costs as well as potentially producing secondary pollution (Beltrán-Flores et al., 2021). Therefore, biological treatment methods (such as bioremediation) have attracted researchers' attentions (Beltrán-Flores et al., 2021). Bioremediation involves the microorganisms such as bacteria and microalgae which degrade pesticides through the metabolic enzymes. Low cost, simple operation, and ecofriendliness are some of the advantages of bioremediation techniques (Zhang et al., 2020). Microalgae, as unicellular photosynthetic microorganisms, have diverse species from 200,000 to 500,000. They absorb nutrients at a high-rate fixation of CO_2 through photosynthesis (Singh and Mishra, 2021). Therefore, using microalgae to eliminate pollutants (such as insecticides) has been considered as an effective method. Moreover, the application of microalgae-based systems in the removal of emerging contaminants can reduce the operation cost compared to other treatment methods (especially physicochemical methods). For instance, the cost of the use of UV photolysis for 4-nonylphenol elimination has been estimated to be around 2.66 €/m^3 of wastewater, compared with the cost of around 1.66 €/m^3 for the application of microalgae-based system (López-Pacheco et al., 2019b). In the presence of algae, organic contaminants can be removed not only through biodegradation and biosorption but also over the photodegradation process.

Microalgae, with excretion biopolymers such as proteins, can increase the photodegradation of organic contaminants (Yang et al., 2018).

In the current study, a green microalgae species (*Chlorella vulgaris*) was selected for wastewater treatment and removal of organic pollutants due to its resistance to low temperatures and effective elimination of nitrogen, phosphorus and organic compounds (Ferro et al., 2019). López-Pacheco et al. (2019a) employed a microalgae-based system, including *Chlorella vulgari*, to treat nejayote and swine wastewater. Up to 80% of levofloxacin was removed with *Chlorella vulgaris* (Xiong et al., 2017a). To improve the microalgae efficiency in the removal of pesticides, the co-culture of algae with other microorganisms (such as bacteria or fungi) have been suggested (Leng et al., 2021, Prosenc et al., 2021). Sewage sludge contains many nutrients and organic substrates (e.g., volatile fatty acids) (Xu et al., 2021), and a high diversity of microorganisms in both phylotypes and functions (Nascimento et al., 2018). Emerging contaminants can partially be removed by biodegradation through activated sludge in a biological wastewater treatment system (Dubey et al., 2021). For instance, around 30% of several emerging contaminants were removed via conventional activated sludge plant (Barret et al., 2012). One of the disadvantages of using microalgae and bacteria for the remediation of pollutants is that the recovery of algae and biomass from treated water is costly (Shi et al., 2014). To address this limitation, biochar can be added to the system to improve immobilization of microorganisms. Biochar is a carbonaceous material which is produced by thermo-chemical conversion of biomass under limited oxygen condition (Wang et al., 2021). Biochar has the great porosity and biocompatibility, which can supply growth matrix for microorganism to promote their biodegradation capability (Ouyang et al., 2021). However, to our knowledge, no study has been reported in the combined application of microbes and biochar for insecticides removal. Apart from that, a comprehensive understanding of biochar-microbe-insecticide interactions is a vital part of the biological studies, which can be carried out by molecular docking analysis (Phong et al., 2021), as considered in the current study.

Thus, this project aimed to remove typical insecticides using an integrated system with the microalgae/bacteria consortium immobilized by biochar. In addition, the removal performance was optimized by response surface methodology (RSM). Furthermore, the molecular docking simulation for catalytic enzyme degradation was conducted.

2. Materials and methods

Chlorella vulgaris was obtained from a photobioreactor in our laboratory (Amin-Azma Research Center), which was run at the illumination intensity around 100±20 μmol photons m^{-2} s^{-1} with light: dark cycles of 12 h:12 h and at room temperature 25±2 °C. Then, before inoculation of photobioreactors, *Chlorella vulgaris* was cultivated in an Erlenmeyer flask comprising BG11 medium. Activated sludge biomass was also obtained from the aerobic sequencing batch reactor in our laboratory. Chlorpyrifos and cypermethrin (Table 1) were purchased from Sigma-Aldrich Co. (Petaling Jaya, Malaysia). Then, stock solutions (1 g L^{-1}) were prepared by separately dissolving the insecticides in distilled water. Biochar (4 mm to 5 mm) was purchased from a local shop which was derived from agricultural wastes.

Table 1: Characteristics of insecticides used in this study

2.1. Experimental setup

Two photobioreactors (Figure 1) with a working volume of 5 L were made of glass (30 cm in depth and 16 cm in diameter). Based on the preliminary experiments, both reactors were inoculated with activated sludge biomass and microalgae with the concentrations of 1 g/L and 1 g/L, respectively, which was similar to the findings of Yang et al. (2018). Furthermore, 25 g/L of biochar was added to the first reactor (microorganisms/biochar reactor) based on the preliminary results which was in line with findings of Mojiri et al. (2021). Synthetic aqueous solution, with the concentration of insecticides at 0.1 mg/L (Huang et al., 2021) to 1.1 mg/L

(Avila et al., 2021), was prepared by dissolving stock solution in the distilled water, and pH of synthetic wastewater adjusted to 7.0 (Mojiri et al., 2020). In both photobioreactors, aeration rate was set at 0.4 L/min (Mojiri et al., 2021) based on the preliminary experiments. Contact time was considered as 24 to 96 h. To mix the solution, a magnetic stirrer was placed at the bottom of both reactors.

Figure 1: Schematic of photoreactors in the study

2.2. Microbial community

At the first day before inoculation process, the bacteria community of activated sewage sludge was analyzed. The soil DNA kit (Omega, USA) was employed for DNA extraction based on the manufacturer's guideline. For amplifying V3-V4 region of bacterial 16S rRNA genes, the primer sets 341F (CCTACGGGNGGCWGCAG) and 805R (GACTACHVGGGTATCTAATCC) were used. The PCR mixture (30 μL) included 15 μL of 2× Taq master Mix, 1 μL of Bar-PCR primer F (10 uM), 1 μL of Primer R (10 uM), Genomic DNA 10–20 ng (Fan et al., 2020). The PCR process was as follow: 3 min at 94 °C; 5 cycles at 94 °C for 30 s, 45 °C for 20 s, 65 °C for 30 s; 20 cycles at 94 °C for 20 s, 20 s at 55 °C, 72 °C for 30 s; and 5 min at 72 °C. DNA was purified via an Agencourt AMPure XP (Becman Coulter, Brea, CA, US). The purified DNA was sequenced by using the MiSeq platform with a MiSeq Reagent Kit v3 (Illumina Inc., San Diego, CA, US), and then taxonomic units (OTUs) was analyzed. The sequences were clustered into OTUs by ARB software (Ludwig, 2004).

2.3. Photocatalysis and biosorption processes

To reveal the relative contribution of direct photocatalysis, batch experiments were conducted in the Erlenmeyer flasks, closed with cotton-wool stoppers, following the method of de Wilt et al. (2016). The flasks (without presence of sludge, algae and biochar) include the solution of

insecticides with the concentration of 0.1 to 1.1 mg/L under illumination intensity around 100±20 μmol photons m^{-2} s^{-1} with light: dark cycles of 12:12 and at room temperature 25±2 ^{o}C. With monitoring the concentrations of insecticides in the solution, the contribution of photocatalysis process in removal of insecticides was revealed.

To monitor the contribution of sorption of insecticides into biomass of microorganisms, batch experiments were conducted in the Erlenmeyer flasks (included dead biomass, autoclaved at 110 °C for 20 min), closed with cotton-wool stoppers, on the basis of Norvill et al. (2017) in a dark place. The flasks include the solution of insecticides with the concentration of 0.1-1.1 mg/L, at room temperature of 25±2 ^{o}C.

2.4. Analytical methods

To measure the concentration of chlorpyrifos and cypermethrin, the high-performance liquid chromatography (HPLC, Agilent, Germany) with the UV detector with the C_{18} column was used. The mobile phase comprised methanol (88%) and ultra-pure water (12%) with a flow rate of 1 mL/min (Sheikhi et al., 2021). The expression 3σ/s ("σ" specifies the standard deviation of the peak and "s" specifies the slope of the corresponding calibration curve) was calculated to determine the limit of detection (LOD) (Mojiri et al., 2021). The autosorb (Quantachrome AS1WinTM-automated gas-sorption apparatus, Boynton Beach, FL, USA) was employed to determine the BET surface of biochar. For biochar, BET surface area, Langmuir surface area , micropore area , and micropore volume were 813 m^2/g, 936 m^2/g, 317 m^2/g, and 0.23 cc/g, respectively.

2.5. Statistical analysis

Equation 1 was applied to estimate the removal effectiveness (%). Response surface methodology (RSM) is considered as a significant tool in designing and optimizing a treatment method. This software makes a study strategy systematic and effective to assess the interactive

effects of several parameters with the help of statistical procedures (Abdulgader et al., 2020). In this study, RSM was applied by the Design Expert Software (Version 10.0) to optimize the abatement effectiveness of insecticides during running both reactors. Contact time (24-96 h ,) (Mojiri et al., 2021) and initial concentrations (0.1-1.1mg/L) (Avila et al., 2021) were considered as the independent parameters at three levels: low (−1), central (0), and high (+1) (Bashir et al., 2010). A quadratic model (equation 2), including the linear model, was applied, where values of P is < 0.05 (Dolatabadi and Ahmadzadeh, 2019). All experiments were conducted in triplicate.

$$Removal\ (\%) = \left(\frac{Initial\ concentration\ of\ pesticides - Final\ concentration\ of\ pesticides}{Initial\ concentration\ of\ pesticides} \right) 100 \quad (1)$$

$$Y = \beta_0 + \sum_{i=1}^{n} \beta_i X_i + \sum_{i=1}^{n} \beta_{ii} X_i^2 + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} \beta_{ij} X_i X_j \quad (2)$$

where removal efficiency is denoted by *Y*; the linear coefficient and the quadratic are specified by β_i and β_{ii}; β_{ij} signifies the interaction coefficient; and X_i and X_j demonstrate the independent coded variables (Dolatabadi and Ahmadzadeh, 2019).

2.6. Insecticides toxicity to *Chlorella vulgaris*

The tests on the toxicity effects of insecticides on *Chlorella vulgaris* were carried out in the Erlenmeyer flasks (with 5×10^4 cells mL^{-1} of microalgae) with different concentrations of insecticides (0.0 mg/L to 4.0 mg/L) and contact time (0 to 72 h) (Vagi et al., 2018). The UV–Vis spectrophotometer (UV-1280, Shimadzu, Japan) was employed to measure chlorophyll and protein content. To prepare the samples for tests, 10 mL of culture was gathered by centrifugation at 4500 rpm for 15 min (Xiong et al., 2017b). Samples were analyzed on three wavelengths 663 nm and 646 nm, and then the chlorophyll contents were calculated by equations 3 to 5 (Damergi et al., 2017).

Chlorophyll a (µg/mL) = 12.25 (A_{663}) – 2.55 (A_{646}) (3)

Chlorophyll b (µg/mL) = 20.13 (A_{646}) - 4.91 (A_{663}) (4)

Chlorophyll a+b (µg/mL) = 17.76 (A_{646}) + 7.34 (A_{663}) (5)

where A_{663} and A_{646} indicate the absorbance at the wavelength of 663 nm and 646 nm, respectively.

To monitor the protein content, samples were analyzed on two wavelengths 280 nm and 260 nm, and then the protein content was calculated by equation 6 (Jabir et al., 2021).

$$Protein\ content = (1.55 \times A_{280}) - (0.77 \times A_{260}) \quad (6)$$

where A_{280} and A_{260} are the absorbance at the wavelength of 280 nm and 260 nm, respectively.

Specific growth rate (μ) was evaluated by equation 7:

$$\mu = \frac{N_t - N_0}{t - t_0} \quad (7)$$

where μ is stated as the logarithmic increase in cell density per unit time, N_t and N_0 define the cell number at specified time (t) and starting time (t_0), respectively.

Based on the scheduled time (0, 24, 48 and 72 hours), cell densities were estimated by the microscope with the improved Neubauer hemocytometer (Vagi et al., 2018). Then, the growth inhibition (I) with comparing the growth rate of microalgae ($\mu_{insecticides}$) in the presence of insecticides and microalgae in control (μ_{blank}) condition (without presence of insecticides) was calculated by equation 8.

$$I(\%) = \frac{(\mu_{control} - \mu_{insecticides})100}{\mu_{control}} \quad (8)$$

2.7. Bioinformatic analysis and protein preparation

2.7.1. Software

Python language was downloaded from www.python.com, Molecular graphics laboratory (MGL) tools was downloaded from http://mgltools.scripps.edu and AutoDock4.2 was downloaded from http://autodock.scripps.edu, Bio Via draw was downloaded from http://accelrys.com, Discovery studio visualizer 2017 was downloaded from

http://accelrys.com , and Chem3D was downloaded from https://acms.ucsd.edu (Venkatesan et al., 2010).

2.7.2. Methods

The three dimensional crystal structure of hydrolase enzymes in Bacteroide with PDB ID: 5JOW, isochorismatase hydrolase protein in proteobacteria with PDB ID: 3LQY and carboxypeptidase protein in Vulgaris with PDB ID: 6T9Y were selected and downloaded from Protein Data Bank (www.rvcsb.org/pdb) (Figure 2) (Fang et al., 2013). The complexes bound to the receptor molecule, all the non-essential water molecules and heteroatoms were deleted and ultimately hydrogen atoms were added to the target receptor molecule using Argus Lab.

Figure 2: Vug. carboxypeptidase in microalgae with PDB ID (6T9Y), Pro. isochorismatase hydrolase in *Proteobacteria* with PDB ID (3LQY) and Bac. alpha-L-arabinofuranosidase in *Bacteroide* with PDB ID (5JOW).

2.7.3. Ligand preparation

Ligand compounds available with identified structure were downloaded from Pubchem to make sdf format and converted to PDB format using Pymol, and further used for docking studies (Maiti and Banerjee, 2021). The starting structures of the proteins were prepared using AutoDock tools. Water molecule was deleted, polar hydrogen and Kollman charges were added to the protein starting structure (Atif et al., 2021). Grid box was set with the size of 126×126×126 Å with the grid spacing of 0.375 Å at the binding site. Gasteiger charges were assigned into optimized ligand using AutodockTools. 100 docking runs were conducted with the mutation rate of 0.02 and the crossover rate of 0.8. The population size was set to use 150 randomly placed individual. Lamarckian Genetic algorithm was used as the searching

algorithm with a translational step of 0.2 Å, a quaternion step of 5 Å and a torsion step of 5 Å. Most populated and lowest binding free energy (Badroon et al., 2020).

2.8. Adsorption isotherm

The adsorption isotherm experiments were carried out at room temperature (25 °C) in beakers (250 mL) which contained 0.32 mg/L of insecticides and biochar (0-25 g/L), with the pH adjusted to 7.0. The mixtures were shaken for 24 h at 200 rpm to reach equilibrium. The capacity of adsorption (mg/g) was calculated by equation 9.

$$q_e = \frac{(C_0 - C_{eq})V}{m_s} \qquad (9)$$

where C_0 and C_{eq} denote the concentration of insecticides (mg/L) at time 0 and at equilibrium, and V and m_s are volume (L) of solution and mass of adsorbent (g), respectively.

3. Results and discussion

First, two photobioreactors were employed to remove chlorpyrifos and cypermethrin from aqueous solution. The first reactor included biochar, *Chlorella vulgaris*, and activated sludge; the second reactor included *Chlorella vulgaris* and activated sludge only. The concentrations of insecticides varied from 0.1 mg/L to 1.1 mg/L, and contact time ranged from 24 h to 96 h. The removal performance of reactors 1 and 2 is shown in Table 2 (Tables A.1 and A.2, in the supplementary file show the removal efficiency), respectively. In addition, the toxicity of insecticides to algae, and adsorption isotherm studies of insecticides removal by biochar were conducted in this study.

Table 2: Experimental design and results (Reactors 1 and 2)

3.1. Removal of chlorpyrifos and cypermethrin from synthetic wastewater

As shown in Table 2 and Figure 3, the maximum removal of CPF and CYP in reactor 1 was 87.2% (0.436 mg/L) and 93.4% (0.467 mg/L), respectively, where the contact time reached 72 h and initial concentration of insecticides was 0.5 mg/L. The minimum abatement of CPF and CYP for reactor 1 was 62.5% (0.688 mg/L) and 63.9% (0.703 mg/L), respectively, where the contact time reached 24 h and initial concentration of insecticides was 1.1 mg/L.

Figure 3: Surface plots for the removal of (a) CPF, and (b) CYP, in reactor 1

As indicated in Table 2 and Figure 4, the maximum elimination of CPF and CYP for reactor 2 was 67.3% (0.202 mg/L) and 69.1% (0.207 mg/L), respectively, with the contact time of 72 h and initial concentration of insecticides of 0.3 mg/L. The minimum removal of CPF and CYP for reactor 2 was 39.9% (0.439 mg/L) and 40.8% (0.449 mg/L), where the contact time was 24 h and initial concentration of insecticides was 1.1 mg/L. It is obvious that the performance of reactor 1 in removal of insecticides was significantly higher than the reactor 2.

Figure 4: Surface plots for removal of (a) CPF, and (b) CYP in reactor 2

Avila et al. (2021) removed 74% of CYP by using microalgae consortium, with 0.1 mg/L of initial CYP concentration and contact time of 7 days. Matamoros and Rodríguez (2016) reported that using green microalgae could improve the removal efficiency of CPF by 50%, where initial concentration of pesticides was 10 µg/L and hydraulic retention time was 2 to 8 days. Ferrando and Matamoros (2020) removed around 36% of organic micropollutants from groundwater by microalgae-based system. The maximum removal rate of CPF and CYP with both reactors was more than in comparison to other studies, demonstrating the advantages of using microalgae and activated sludge consortium in this study. Mailler et al. (2014) removed around 60% of hydrophobic and volatile compounds by activated sludge process. The growth

rate and microalgae activities could be increased in presence of the bacteria (Nguyen et al., 2019), which may increase the removal of insecticides with microalgae though our study.

As shown in Figure 5, *Proteobacteria*, *Bacteroidetes* and *Chloroflexi* were the dominant bacteria in the activated sludge. Similarly, Zhang et al. (2019) reported that *Proteobacteria*, *Bacteroidetes* and *Chloroflexi* were dominant bacteria in a wastewater treatment plant. Meng et al. (2021) stated that *Proteobacteria* and *Bacteroidetes* could remove the emerging pollutants; however, high concentration of organic pollutants could affect the bacteria performance in bioremediation. Modrzyński et al. (2021) removed up to 50% of sulfamethoxazole with using bioremediation method, where *Actinobacteria* and *Chloroflexi* accounted for 21% and 11% of microbial community. During remediation of pesticides, ammonia is produced which may have a negative effect on the performance of the system. For instance, Huete-Ortega et al. (2018) expressed that high concentration of ammonia had a negative effect on the lipid productivity of some microalgae. Guo et al. (2020) stated that *Proteobacteria*, *Bacteroidetes, Chloroflexi,* and *Nitrospirae* were the main contributors in ammonia removal of high concentration. Therefore, the negative effects of high concentration of ammonia on the microbial consortium would be decreased in this study due to the presence of activate sludge.

Figure 5: Microbial community of activated sludge used in this study

As shown by Table 2, the removal performance of reactor 1 was more than the reactor 2, which can be attributed to biochar in the reactor 1. Nie et al. (2020) expressed that the immobilization of microalgae is an effective approach in improving removal of pesticides by algae-based system. For example, Ferrando and Matamoros (2020) improved the removal of emerging contaminant from 36% to 51% by immobilized microalgae-based method. Moreover, Mojiri et al. (2020) stated that applying biochar in biological treatment methods was not only improving

microbial community by immobilization of microorganisms and balancing nutrient content, but also served as an adsorbent for removing contaminants.

Drouin et al. (2021) and de Wilt et al. (2016) reported that while biodegradation is the main pathway of pesticide degradation in the environment, photodegradation and biosorption also have a vital role in removal process. In this study, the direct photocatalysis process removed between 11% (contact time 24 h, initial insecticide concentration of 1.1 mg/L) and 19% (contact time 96 h, initial insecticide concentration of 0.4 mg/L) of contaminants. Pesticide molecules can absorb light, resulting in bond cleavage, during direct photodegradation (Drouin et al., 2021). Drouin et al. (2021) reported that the photodegradation of some pesticides was three times lower than the dissolved organic matters. de Wilt et al. (2016) expressed that sorption to algal biomass usually accounted for less than 20% of the emerging contaminants removal. The minimum and maximum sorption were 16% (contact time 24 h and concentration 0.9 mg/L) to 21% (contact time 96 h and concentration 0.1 mg/L) during biosorption experiments in this study. On the other hand, the contributions of photodegradation and biosorption in removal of insecticides were less than 22%, and the most of the insecticides were removed by biodegradation process. de Wilt et al. (2016) stated that biodegradation is the main mechanism of emerging contaminants removal.

RSM and central composite design (CCD) were applied to optimize the abatement performance of both reactors in this study. The R^2 plots of insecticides elimination, predicted versus actual data, with both reactors are presented in Figures A.1 and A.2 (supplementary file). The R^2 (for actual data) and predicted R^2 were higher than 0.9, displaying that the RSM could optimize the performance of reactors (Khalid et al., 2019). The statistical analysis of optimization of both reactors' performance is shown in Table 3. On the basis of the actual results and significant results at $p < 0.5$, the final equations for the elimination of CPF and CYP with the reactors 1 and 2 are shown in equations 10-13. Based on the RSM, the maximum removal of CPF and CYP with reactor 1 was 88.80% and 93.12% at the optimum contact time (69.7 h) and 0.32

mg/L of initial concentration. The maximum elimination of CPF and CYP with reactor 2 was 66.96% and 64.44%, respectively, at the optimum contact time (71.8 h) and 0.29 mg/L of initial concentration.

For reactor 1:

$$69.62 + 86.10B - 176.65B^2 + 83.96B^3 \quad (10)$$

$$67.56 + 150.06B - 291.90B^2 + 139.95B^3 \quad (11)$$

For reactor 2:

$$56.76 + 5.82A - 21.78B - 6.01B^2 - 6.93A^3 + 13.19B^3 \quad (12)$$

$$62.10 + 72.38B + 0.002B^2 + 73.17B^3 \quad (13)$$

where A and B define the contact time (h) and initial concentrations of CPF and CYP, respectively.

Table 3: ANOVA and optimization results

3.2. Toxicity of chlorpyrifos and cypermethrin on *Chlorella vulgaris*

The effects of toxic pollutants such as insecticides on algae can affect their structure and function, resulting in reducing the performance of algae in removal of pollutants, and biomass productivity (Satyavani et al., 2012). Therefore, the toxic effects of insecticides (0-4.0 mg/L) on *Chlorella vulgaris* were examined under different contact time from 0 to 72 h.

As shown in Figure 6a, the exposure to insecticide concentrations from 0 to 1.5 mg/L for 24 h increased the chlorophyll content from 8.7 to 10.1 mg/L. However, the chlorophyll content decreased from 12.9 to 2.0 mg/L with increasing the insecticide concentrations from 0.5 to 4.0 mg/L in contact time 72 h. On the other hand, the exposure to low concentrations of insecticides in a short time (> 24 h) could increase the chlorophyll content, and with increasing contact time and concentration of insecticides the chlorophyll decreased. Delorenzo et al. (2001) reported an increase in content of chlorophyll *a* with exposure to chlorpyrifos (10 pg/L). Bighiu et al.

(2020) stated that an increase in chlorophyll content in response to a biotic or abiotic stress is considered as a mechanism of shade adaptation, named "greening effect" in algae. An increase in photosynthetic efficiency might indicate hormesis, with biofilm compensating for the pesticides-induced disturbance (Bighiu et al., 2020). Mostafa and Helling (2002) reported that the chlorophyll content reduced to 9.5% of normal conditions (algae in the control tests) with increasing the pesticides concentrations to >0.81 mg/L and contact time more than 72 hours, which is in line with the findings here. Mojiri et al. (2021) expressed that reduction of chlorophyll contents in microalgae exposed to organic pollutants might be explained with the reactive oxygen species (ROS)-mediated damage to the photosystem and chlorophyll biosynthesis. Chlorophyll of cells might be used as a protective technique to decrease the accumulated ROS in chloroplasts. Apart from that, Mostafa and Helling (2002) stated that the reduction in the chlorophyll contents leads to alter the photosynthetic unit size and the concentration of photochemical reaction centers of the photosystems.

Based on Figure 6b, the protein content increased from 37.3% at time zero to 56.7% after 72 h during exposure to insecticide concentration of 0.5 mg/L. Additionally, the minimum protein content (16.7%) was recorded in contact time 72 h and insecticide concentration of 4 mg/L. On the other hand, low pesticide concentration in a short time might increase the protein content, which may be attributed to increasing chlorophylls and photosynthesis of algae. The exposure to low concentrations of pesticides in a short time increased the protein content in *Chlorella pyrenoidosa*, which then decreased with increasing the concentration and contact time (Nong et al., 2021). Dai et al. (2021) reported that protein content of *Chlorella vulgaris* significantly reduced by exposure to 1.05 mg/L triclosan. Nong et al. (2021) suggested two reasons for reduced protein content: (1) a decrease in chlorophylls with increasing the concentration of pesticides and contact time must have a great effect on protein production; and (2) increasing concentration of pesticides may block the metabolism of algae which result in protein degradation.

As indicated in Figure 6c, no growth inhibition was found in low concentrations of insecticides (0.5 mg/L), and short contact time (>24 h). Maximum inhibition (89.7%) was reached at insecticides concentration of 4 mg/L and contact time of 72 h. Vagi et al. (2018) reported a significant reduction in growth of microalgae exposed to 2.00-3.00 mg/L organophosphorus insecticide, which is in agreement with the current study. DeLorenzo et al. (2001) reported that growth rate of *Anabaena doliolum* completely reduced with increasing endosulfa concentration more than the 3 mg/L. Organic contaminant may prevent the normal mitotic divisional process in microalgae, which reduces the growth rate of microalgae (DeLorenzo et al., 2001). Liu et al. (2019) stated that increasing the pesticides concentrations and contact time could inhibit the growth of *Chlorella vulgaris*, as the exposure to pesticides for 96 h reduced the growth rate of *Chlorella vulgaris* for more than 23%.

Figure 6: Toxicity of insecticides on *Chlorella vulgaris*, as shown in (a) total chlorophyll, (b) protein content, and (d) inhibition rate.

3.3. Molecular docking

The free binding energy of cypermethrin after interaction with Vug was -7.03Kcal/mol. It showed two strong hydrogen bonds with ARG189 and SER188 via oxygen linked to carbonyl group and triple bonded nitrogen of cypermethrin (Figure 7). The interaction of cypermethrin with Pro showed three hydrogen bonds with TYR18, ASN27 and THR122 (Figure 8). These bonds occurred between etheric oxygen and oxygen linked to carbonyl group and residues in Pro. Moreover, the interaction between Bac and cypermethrin showed three hydrogen bonds with free binding energy of -10.21 kcal/mol. It showed that the triple bonded nitrogen and oxygen linked to carbonyl group are involved in interaction with TYR271 and LEU211. This study showed the possibility of synergistic effects of three enzymes in degradation of cypermethrin would be more successful than working individually (Table 4).

Figure 7: Chemical interactions between Vug and ligands

Figure 8: Chemical interactions between Pro and ligands

Figure 9: Chemical interactions between Bac and ligands

The same molecular docking studies using chlorpyrifos showed the lowest free binding energy happened in interaction with Bac (Figure 9). It demonstrated three conventional hydrogen bonds with LEU211 and ALA239 via the oxygen linked to phosphate group. The interaction between Pro and chlorpyrifos demonstrated four hydrogen bonds with TYR18, LYS92, ALA120 and ILE126 via sulfur and chloride group of chlorpyrifos. The result of molecular docking between Vug and chlorpyrifos also demonstrated three hydrogen bonds with ILE197, HIS269 and LYS198 to sulfur and oxygen linked to phosphate groups. Hydrogen bonds in this study showed the stability of ligand-enzyme interactions. The smaller the binding energy between enzyme and substrate is, the more favorable it is for enzyme to combine with substrate, and the easier pollutants can be degraded (Hongyan et al., 2019). Molecular docking studies using the selected catalytic enzymes in *Proteobacteria*, *Bacteroidetes* and algae suggests that probably the use of three organisms can show better efficacy in the degradation of cypermethrin and chlorpyrifos.

Table 4: Free binding energy (Fbe) and inhibition constant of selected proteins with ligands

3.4. Biochar adsorption of chlorpyrifos and cypermethrin in synthetic wastewater

The characteristics of biochar showed that BET surface area, Langmuir surface area , micropore area, and micropore volume were 818 m^2/g, 981 m^2/g, 413 m^2/g, and 0.22 cc/g, respectively. Apart from improving the microbial community with using biochar, biochar is a carbon-rich material that is frequently employed as an adsorbent for pollutant elimination (Suo

et al., 2019). Therefore, adsorption isotherm studies were conducted to monitor the removal of pesticides by biochar. The key data from the Langmuir and Freundlich models are shown in Table 5. The Langmuir isotherm (equation 14) can effectively justify the monolayer adsorption (Mojiri et al., 2020b). Baharum et al. (2020) reported *b* (0.20), R^2 (0.89) and Q_m (4.22 mg/g) for pesticide (diazinon) removal with biochar (extracted from coconut shell), which are near our findings (Q_m = 5.2 to 6.32 mg/g, b = 0.17 to 0.21, and R^2 = 0.911 to 0.937). Based on the R^2 values, the Langmuir model explain well the adsorption of CPF and CPY with biochar.

$$\frac{x}{m} = \frac{abC_e}{1+bC_e} \qquad (14)$$

where the mg of adsorbate per g of adsorbent is presented by x/m, the constants of Langmuir model are *a* and *b*, and the equilibrium concentration of adsorbate (mg/L) is shown by C_e.

The Freundlich isotherm (equation 15) is frequently applied to define adsorption characteristics in for heterogeneous surfaces.

$$\frac{x}{m} = K_f C_e^{\frac{1}{n}} \qquad (15)$$

where the adsorption capacity of the adsorbent (mg/g $(L/mg)^{1/n}$) and intensity of adsorption are defined by K_f and n, respectively.

Zhao et al. (2018) reported K_f of 0.6 for removal of pesticide imidacloprid with biochar (peanut shell), which is similar to our findings (K_f 0.23 to 34 (mg/g $(L/mg)^{1/n}$)). Moreover, CFP removal by biochar could be explained by the Freundlich model due to R^2 of0.93. The related graphs for the Langmuir and Freundlich models are shown in Figures A.3 and A.4 (supplementary file).

Table 5: The Freundlich and Langmuir isotherms constants

Conclusions

CPF and CYP were eliminated by two photobioreactors, with the first reactor containing biochar, *Chlorella vulgaris* and activated sludge, and the second reactor containing *Chlorella vulgaris* and activated sludge only. DNA extraction showed that the *Proteobacteria*, *Bacteroidetes* and *Chloroflexi* were the dominant phyla of the activated sludge. In addition, the interaction between three hydrolase proteins in three different microorganisms, and CYP as well as CPF were simulated individually by molecular docking. The simultaneous use of microorganisms was suggested for further studies to be more successful in the degradation of ligands. The main findings of the study are listed below:

1. The integrated biochar/microbial system eliminated 87.2% of CPF and 93.4% of CYP and, which are higher than 67.3% of CPF and 69.1% of CYP in the absence of biochar.
2. Based on the RSM, the maximum abatement of CPF and CYP with reactor 1 was 88.80% and 93.12%, respectively, at the optimum contact time of 69.7 h and initial insecticide concentration of 0.32 mg/L.
3. The maximum chlorophyll content and protein were 12.9 mg/L and 56.7%, which decreased with increasing concentration of insecticides and contact time.
4. Both Freundlich and Langmuir models could be fitted to CPF and CYP adsorption by biochar with high R^2 values.

Acknowledgements

Authors would like to express their gratitude to the Amin-Azma Research Institute for providing facilities for doing this research.

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

Authorship contribution statement:

Amin Mojiri: Conceptualization, Methodology, Software, Writing - original draft. **John L. Zhou:** Writing – original draft & editing. **Mansoureh Nazari V.:** Writing – original draft, Software. **Shahabaldin Rezania**: Writing - review & editing; **Hossein Farraji:** Methodology and Investigation. **Mohammadtaghi Vakili:** Investigation.

References

Abdulgader, M., Yu, Q.J., Zinatizadeh, A.A., Williams, P., Rahimi, Z., 2020. Application of response surface methodology (RSM) for process analysis and optimization of milk processing wastewater treatment using multistage flexible fiber biofilm reactor. J. Environ. Chem. Eng. 8, 103797. https://doi.org/10.1016/j.jece.2020.103797

Ali, H.F.H., El-Sayed, N.M., Khodeer, D.M., Ahmed, A.A.M., Hanna, P.A., Moustafa, Y.M.A., 2020. Nano selenium ameliorates oxidative stress and inflammatory response associated with cypermethrin-induced neurotoxicity in rats. Ecotoxicol. Environ. Saf. 195, 110479. https://doi.org/10.1016/j.ecoenv.2020.110479

Ali, I., Alharbi, O.M.L., ALOthman, Z.A., Al-Mohaimeed, A.M., Alwarthan, A., 2019. Modeling of fenuron pesticide adsorption on CNTs for mechanistic insight and removal in water. Environ. Res. 170, 389–397. https://doi.org/10.1016/j.envres.2018.12.066

Atif, M., Nazari, M.V., Ahmed, M.B.K., Aslam, M., Majid, A.S.A., Iqbal, M.A., 2021. Docking-designed Green Synthesis and In-Vitro Anticancer Studies of New Binuclear Se-N-Heterocyclic Carbene Adducts and their Azolium Salts. Appl. Biochem. Biotechnol. 41. https://doi.org/10.21203/rs.3.rs-678554/v1

Avila, R., Peris, A., Eljarrat, E., Vicent, T., Blánquez, P., 2021. Biodegradation of hydrophobic pesticides by microalgae: Transformation products and impact on algae biochemical methane potential. Sci. Total Environ. 754, 142114. https://doi.org/10.1016/j.scitotenv.2020.142114

Badroon, N., Abdul Majid, N., Al-Suede, F., Nazari V., M., Giribabu, N., Abdul Majid, A.,

Eid, E., Alshawsh, M., 2020. Cardamonin Exerts Antitumor Effect on Human Hepatocellular Carcinoma Xenografts in Athymic Nude Mice through Inhibiting NF-κβ Pathway. Biomedicines 8, 586. https://doi.org/10.3390/biomedicines8120586

Baharum, N.A., Nasir, H.M., Ishak, M.Y., Isa, N.M., Hassan, M.A., Aris, A.Z., 2020. Highly efficient removal of diazinon pesticide from aqueous solutions by using coconut shell-modified biochar. Arab. J. Chem. 13, 6106–6121. https://doi.org/10.1016/j.arabjc.2020.05.011

BARRET, M., DELGADILLO-MIRQUEZ, L., TRABLY, E., DELGENES, N., BRAUN, F., CEA-BARCIA, G., STEYER, J.P., PATUREAU, D., 2012. Anaerobic Removal of Trace Organic Contaminants in Sewage Sludge: 15 Years of Experience. Pedosphere 22, 508–517. https://doi.org/10.1016/S1002-0160(12)60035-6

Bashir, M.J.K., Aziz, H.A., Yusoff, M.S., Aziz, S.Q., Mohajeri, S., 2010. Stabilized sanitary landfill leachate treatment using anionic resin: Treatment optimization by response surface methodology. J. Hazard. Mater. 182, 115–122. https://doi.org/10.1016/j.jhazmat.2010.06.005

Beltrán-Flores, E., Sarrà, M., Blánquez, P., 2021. Pesticide bioremediation by Trametes versicolor: Application in a fixed-bed reactor, sorption contribution and bioregeneration. Sci. Total Environ. 794, 148386. https://doi.org/10.1016/j.scitotenv.2021.148386

Bighiu, M.A., Gottschalk, S., Arrhenius, Å., Goedkoop, W., 2020. Pesticide Mixtures Cause Short-Term, Reversible Effects on the Function of Autotrophic Periphyton Assemblages. Environ. Toxicol. Chem. 39, 1367–1374. https://doi.org/10.1002/etc.4722

Bilal, M., Iqbal, H.M.N., Barceló, D., 2019. Persistence of pesticides-based contaminants in the environment and their effective degradation using laccase-assisted biocatalytic systems. Sci. Total Environ. 695, 133896. https://doi.org/10.1016/j.scitotenv.2019.133896

Dai, Z., Luo, X., Yang, A., Wang, J., Fu, H., Wu, Y., 2021. The Effects of Triclosan on

Physiological and Photosynthetic Characteristics of Chlorella vulgaris. Water 13, 1355. https://doi.org/10.3390/w13101355

Damergi, E., Schwitzguébel, J.-P., Refardt, D., Sharma, S., Holliger, C., Ludwig, C., 2017. Extraction of carotenoids from Chlorella vulgaris using green solvents and syngas production from residual biomass. Algal Res. 25, 488–495. https://doi.org/10.1016/j.algal.2017.05.003

de Wilt, A., Butkovskyi, A., Tuantet, K., Leal, L.H., Fernandes, T. V., Langenhoff, A., Zeeman, G., 2016. Micropollutant removal in an algal treatment system fed with source separated wastewater streams. J. Hazard. Mater. 304, 84–92. https://doi.org/10.1016/j.jhazmat.2015.10.033

DeLorenzo, M.E., Scott, G.I., Ross, P.E., 2001. Toxicity of pesticides to aquatic microorganisms: A review. Environ. Toxicol. Chem. 20, 84–98. https://doi.org/10.1002/etc.5620200108

Ding, J., Bu, L., Cui, B., Zhao, G., Gao, Q., Wei, L., Zhao, Q., Dionysiou, D.D., 2020. Assessment of solar-assisted electrooxidation of bisphenol AF and bisphenol A on boron-doped diamond electrodes. Environ. Sci. Ecotechnology 3, 100036. https://doi.org/10.1016/j.ese.2020.100036

Dolatabadi, M., Ahmadzadeh, S., 2019. A rapid and efficient removal approach for degradation of metformin in pharmaceutical wastewater using electro-Fenton process; optimization by response surface methodology. Water Sci. Technol. 80, 685–694. https://doi.org/10.2166/wst.2019.312

Drouin, G., Droz, B., Leresche, F., Payraudeau, S., Masbou, J., Imfeld, G., 2021., 2021. Direct and indirect photodegradation of atrazine and S-metolachlor in agriculturally impacted surface water and associated C and N isotope fractionation. ChemRxiv. https://doi.org/10.33774/chemrxiv-2021-sg66n

Dubey, M., Mohapatra, S., Tyagi, V.K., Suthar, S., Kazmi, A.A., 2021. Occurrence, fate, and

persistence of emerging micropollutants in sewage sludge treatment. Environ. Pollut. 273, 116515. https://doi.org/10.1016/j.envpol.2021.116515

Fan, J., Chen, Y., Zhang, T.C., Ji, B., Cao, L., 2020. Performance of Chlorella sorokiniana-activated sludge consortium treating wastewater under light-limited heterotrophic condition. Chem. Eng. J. 382, 122799. https://doi.org/10.1016/j.cej.2019.122799

Fang, S., Li, L., Cui, B., Men, S., Shen, Y., Yang, X., 2013. Structural insight into plant programmed cell death mediated by BAG proteins in Arabidopsis thaliana. Acta Crystallogr. Sect. D Biol. Crystallogr. 69, 934–945. https://doi.org/10.1107/S0907444913003624

Ferrando, L., Matamoros, V., 2020. Attenuation of nitrates, antibiotics and pesticides from groundwater using immobilised microalgae-based systems. Sci. Total Environ. 703, 134740. https://doi.org/10.1016/j.scitotenv.2019.134740

Ferro, L., Gojkovic, Z., Muñoz, R., Funk, C., 2019. Growth performance and nutrient removal of a Chlorella vulgaris-Rhizobium sp. co-culture during mixotrophic feed-batch cultivation in synthetic wastewater. Algal Res. 44, 101690. https://doi.org/10.1016/j.algal.2019.101690

Guo, Y., Wang, J., Shinde, S., Wang, X., Li, Y., Dai, Y., Ren, J., Zhang, P., Liu, X., 2020. Simultaneous wastewater treatment and energy harvesting in microbial fuel cells: an update on the biocatalysts. RSC Adv. 10, 25874–25887. https://doi.org/10.1039/D0RA05234E

Hamadeen, H.M., Elkhatib, E.A., Badawy, M.E.I., Abdelgaleil, S.A.M., 2021. Green low cost nanomaterial produced from Moringa oleifera seed waste for enhanced removal of chlorpyrifos from wastewater: Mechanism and sorption studies. J. Environ. Chem. Eng. 9, 105376. https://doi.org/10.1016/j.jece.2021.105376

Hongyan, L., Zexiong, Z., Shiwei, X., He, X., Yinian, Z., Haiyun, L., Zhongsheng, Y., 2019. Study on transformation and degradation of bisphenol A by Trametes versicolor laccase

and simulation of molecular docking. Chemosphere 224, 743–750. https://doi.org/10.1016/j.chemosphere.2019.02.143

Huang, Y., Zhang, W., Pang, S., Chen, J., Bhatt, P., Mishra, S., Chen, S., 2021. Insights into the microbial degradation and catalytic mechanisms of chlorpyrifos. Environ. Res. 194, 110660. https://doi.org/10.1016/j.envres.2020.110660

Huete-Ortega, M., Okurowska, K., Kapoore, R.V., Johnson, M.P., Gilmour, D.J., Vaidyanathan, S., 2018. Effect of ammonium and high light intensity on the accumulation of lipids in Nannochloropsis oceanica (CCAP 849/10) and Phaeodactylum tricornutum (CCAP 1055/1). Biotechnol. Biofuels 11, 60. https://doi.org/10.1186/s13068-018-1061-8

Jabir, T.F., Noor Abbood, H.A., Salman, F.S., Hafit, A.Y., 2021. Influence of pH, pesticide and radiation interactions on the chemical composition of Chlorella vulgaris algae. IOP Conf. Ser. Earth Environ. Sci. 722, 012046. https://doi.org/10.1088/1755-1315/722/1/012046

Kamboh, M.A., Arain, S.S., Jatoi, A.H., Sherino, B., Algarni, T.S., Al-onazi, W.A., Al-Mohaimeed, A.M., Rezania, S., 2021. Green sporopollenin supported cyanocalixarene based magnetic adsorbent for pesticides removal from water: Kinetic and equilibrium studies. Environ. Res. 201, 111588. https://doi.org/10.1016/j.envres.2021.111588

Khalid, A.A.H., Yaakob, Z., Abdullah, S.R.S., Takriff, M.S., 2019. Analysis of the elemental composition and uptake mechanism of Chlorella sorokiniana for nutrient removal in agricultural wastewater under optimized response surface methodology (RSM) conditions. J. Clean. Prod. 210, 673–686. https://doi.org/10.1016/j.jclepro.2018.11.095

Kodali, J., Talasila, S., Arunraj, B., Nagarathnam, R., 2021. Activated Coconut Charcoal as a super adsorbent for the removal of organophosphorous pesticide monocrotophos from water. Case Stud. Chem. Environ. Eng. 3, 100099. https://doi.org/10.1016/j.cscee.2021.100099

Laskowski, D.A., 2002. Physical and Chemical Properties of Pyrethroids. pp. 49–170. https://doi.org/10.1007/978-1-4757-4260-2_3

Leng, L., Li, W., Chen, Jie, Leng, S., Chen, Jiefeng, Wei, L., Peng, H., Li, J., Zhou, W., Huang, H., 2021. Co-culture of fungi-microalgae consortium for wastewater treatment: A review. Bioresour. Technol. 330, 125008. https://doi.org/10.1016/j.biortech.2021.125008

Liu, C., Liu, S., Diao, J., 2019. Enantioselective growth inhibition of the green algae (Chlorella vulgaris) induced by two paclobutrazol enantiomers. Environ. Pollut. 250, 610–617. https://doi.org/10.1016/j.envpol.2019.04.027

López-Pacheco, I.Y., Carrillo-Nieves, D., Salinas-Salazar, C., Silva-Núñez, A., Arévalo-Gallegos, A., Barceló, D., Afewerki, S., Iqbal, H.M.N., Parra-Saldívar, R., 2019a. Combination of nejayote and swine wastewater as a medium for Arthrospira maxima and Chlorella vulgaris production and wastewater treatment. Sci. Total Environ. 676, 356–367. https://doi.org/10.1016/j.scitotenv.2019.04.278

López-Pacheco, I.Y., Salinas-Salazar, C., Silva-Núñez, A., Rodas-Zuluaga, L.I., Donoso-Quezada, J., Ayala-Mar, S., Barceló, D., Iqbal, H.M.N., Parra-Saldívar, R., 2019b. Removal and biotransformation of 4-nonylphenol by Arthrospira maxima and Chlorella vulgaris consortium. Environ. Res. 179, 108848. https://doi.org/10.1016/j.envres.2019.108848

Ludwig, W., 2004. ARB: a software environment for sequence data. Nucleic Acids Res. 32, 1363–1371. https://doi.org/10.1093/nar/gkh293

Ma, G., Peng, F., Yang, W., Yan, G., Gao, S., Zhou, X., Qi, J., Cao, D., Zhao, Y., Pan, W., Jiang, H., Jing, H., Dong, G., Gao, M., Zhou, J., Yu, F., Wang, J., 2020. The valuation of China's environmental degradation from 2004 to 2017. Environ. Sci. Ecotechnology 1, 100016. https://doi.org/10.1016/j.ese.2020.100016

Mailler, R., Gasperi, J., Rocher, V., Gilbert-Pawlik, S., Geara-Matta, D., Moilleron, R.,

Chebbo, G., 2014. Biofiltration vs conventional activated sludge plants: what about priority and emerging pollutants removal? Environ. Sci. Pollut. Res. 21, 5379–5390. https://doi.org/10.1007/s11356-013-2388-0

Maiti, S., Banerjee, A., 2021. Epigallocatechin gallate and theaflavin gallate interaction in <scp>SARS-CoV</scp> -2 spike-protein central channel with reference to the hydroxychloroquine interaction: Bioinformatics and molecular docking study. Drug Dev. Res. 82, 86–96. https://doi.org/10.1002/ddr.21730

Matamoros, V., Rodríguez, Y., 2016. Batch vs continuous-feeding operational mode for the removal of pesticides from agricultural run-off by microalgae systems: A laboratory scale study. J. Hazard. Mater. 309, 126–132. https://doi.org/10.1016/j.jhazmat.2016.01.080

Meng, L., Wang, J., Li, X., Yu, Y., Zhu, Y., 2021. Microbial community and molecular ecological network in the EGSB reactor treating antibiotic wastewater: Response to environmental factors. Ecotoxicol. Environ. Saf. 208, 111669. https://doi.org/10.1016/j.ecoenv.2020.111669

Modrzyński, J.J., Aamand, J., Wittorf, L., Badawi, N., Hubalek, V., Canelles, A., Hallin, S., Albers, C.N., 2021. Combined removal of organic micropollutants and ammonium in reactive barriers developed for managed aquifer recharge. Water Res. 190, 116669. https://doi.org/10.1016/j.watres.2020.116669

Mojiri, A., Baharlooeian, M., Kazeroon, R.A., Farraji, H., Lou, Z., 2020a. Removal of Pharmaceutical Micropollutants with Integrated Biochar and Marine Microalgae. Microorganisms 9, 4. https://doi.org/10.3390/microorganisms9010004

Mojiri, A., Zhou, J., Vakili, M., Van Le, H., 2020b. Removal performance and optimisation of pharmaceutical micropollutants from synthetic domestic wastewater by hybrid treatment. J. Contam. Hydrol. 235, 103736. https://doi.org/10.1016/j.jconhyd.2020.103736

Mojiri, A., Zhou, J.L., Robinson, B., Ohashi, A., Ozaki, N., Kindaichi, T., Farraji, H., Vakili, M., 2020c. Pesticides in aquatic environments and their removal by adsorption methods. Chemosphere 253, 126646. https://doi.org/10.1016/j.chemosphere.2020.126646

Morsi, R., Bilal, M., Iqbal, H.M.N., Ashraf, S.S., 2020. Laccases and peroxidases: The smart, greener and futuristic biocatalytic tools to mitigate recalcitrant emerging pollutants. Sci. Total Environ. 714, 136572. https://doi.org/10.1016/j.scitotenv.2020.136572

Mostafa, F.I.Y., Helling, C.S., 2002. IMPACT OF FOUR PESTICIDES ON THE GROWTH AND METABOLIC ACTIVITIES OF TWO PHOTOSYNTHETIC ALGAE. J. Environ. Sci. Heal. Part B 37, 417–444. https://doi.org/10.1081/PFC-120014873

Nascimento, A.L., Souza, A.J., Andrade, P.A.M., Andreote, F.D., Coscione, A.R., Oliveira, F.C., Regitano, J.B., 2018. Sewage Sludge Microbial Structures and Relations to Their Sources, Treatments, and Chemical Attributes. Front. Microbiol. 9. https://doi.org/10.3389/fmicb.2018.01462

Nguyen, T.D.P., Nguyen, D.H., Lim, J.W., Chang, C.-K., Leong, H.Y., Tran, T.N.T., Vu, T.B.H., Nguyen, T.T.C., Show, P.L., 2019. Investigation of the Relationship between Bacteria Growth and Lipid Production Cultivating of Microalgae Chlorella Vulgaris in Seafood Wastewater. Energies 12, 2282. https://doi.org/10.3390/en12122282

Nie, J., Sun, Y., Zhou, Y., Kumar, M., Usman, M., Li, J., Shao, J., Wang, L., Tsang, D.C.W., 2020. Bioremediation of water containing pesticides by microalgae: Mechanisms, methods, and prospects for future research. Sci. Total Environ. 707, 136080. https://doi.org/10.1016/j.scitotenv.2019.136080

Nong, Q.-Y., Liu, Y.-A., Qin, L.-T., Liu, M., Mo, L.-Y., Liang, Y.-P., Zeng, H.-H., 2021. Toxic mechanism of three azole fungicides and their mixture to green alga Chlorella pyrenoidosa. Chemosphere 262, 127793. https://doi.org/10.1016/j.chemosphere.2020.127793

Norvill, Z.N., Toledo-Cervantes, A., Blanco, S., Shilton, A., Guieysse, B., Muñoz, R., 2017.

Photodegradation and sorption govern tetracycline removal during wastewater treatment in algal ponds. Bioresour. Technol. 232, 35–43. https://doi.org/10.1016/j.biortech.2017.02.011

Ouyang, X., Yin, H., Yu, X., Guo, Z., Zhu, M., Lu, G., Dang, Z., 2021. Enhanced bioremediation of 2,3′,4,4′,5-pentachlorodiphenyl by consortium GYB1 immobilized on sodium alginate-biochar. Sci. Total Environ. 788, 147774. https://doi.org/10.1016/j.scitotenv.2021.147774

Phong, N.V., Oanh, V.T., Yang, S.Y., Choi, J.S., Min, B.S., Kim, J.A., 2021. PTP1B inhibition studies of biological active phloroglucinols from the rhizomes of Dryopteris crassirhizoma: Kinetic properties and molecular docking simulation. Int. J. Biol. Macromol. 188, 719–728. https://doi.org/10.1016/j.ijbiomac.2021.08.091

Prosenc, F., Piechocka, J., Škufca, D., Heath, E., Griessler Bulc, T., Istenič, D., Buttiglieri, G., 2021. Microalgae-based removal of contaminants of emerging concern: Mechanisms in Chlorella vulgaris and mixed algal-bacterial cultures. J. Hazard. Mater. 418, 126284. https://doi.org/10.1016/j.jhazmat.2021.126284

Satyavani, G., Chandrasehar, G., Varma, K.K., Goparaju, A., Ayyappan, S., Reddy, P.N., Murthy, P.B., 2012. Toxicity Assessment of Expired Pesticides to Green Algae Pseudokirchneriella subcapitata. ISRN Toxicol. 2012, 1–10. https://doi.org/10.5402/2012/247072

Sheikhi, S., Dehghanzadeh, R., Maryamabadi, A., Aslani, H., 2021. Chlorpyrifos removal from aqueous solution through sequential use of coagulation and advanced oxidation processes: By-products, degradation pathways, and toxicity assessment. Environ. Technol. Innov. 23, 101564. https://doi.org/10.1016/j.eti.2021.101564

Shi, J., Podola, B., Melkonian, M., 2014. Application of a prototype-scale Twin-Layer photobioreactor for effective N and P removal from different process stages of municipal wastewater by immobilized microalgae. Bioresour. Technol. 154, 260–266.

https://doi.org/10.1016/j.biortech.2013.11.100

Singh, V., Mishra, V., 2021. Exploring the effects of different combinations of predictor variables for the treatment of wastewater by microalgae and biomass production. Biochem. Eng. J. 174, 108129. https://doi.org/10.1016/j.bej.2021.108129

Suo, F., You, X., Ma, Y., Li, Y., 2019. Rapid removal of triazine pesticides by P doped biochar and the adsorption mechanism. Chemosphere 235, 918–925. https://doi.org/10.1016/j.chemosphere.2019.06.158

Tulun, Ş., Akgül, G., Alver, A., Çelebi, H., 2021. Adaptive neuro-fuzzy interference system modelling for chlorpyrifos removal with walnut shell biochar. Arab. J. Chem. 14, 103443. https://doi.org/10.1016/j.arabjc.2021.103443

Vagi, M.C., Petsas, A.S., Pavlaki, M.D., Smaragdaki, N.M., Kostopoulou, M.N., 2018. Toxic Effects of the Organophosphorus Insecticide Fenthion on Growth and Chlorophyll Production Activity of Unicellular Marine Microalgae Tetraselmis suecica: Comparison between Observed and Predicted Endpoint Toxicity Data, in: Insecticides - Agriculture and Toxicology. InTech. https://doi.org/10.5772/intechopen.72321

Venkatesan, S.K., Shukla, A.K., Dubey, V.K., 2010. Molecular docking studies of selected tricyclic and quinone derivatives on trypanothione reductase of Leishmania infantum. J. Comput. Chem. NA-NA. https://doi.org/10.1002/jcc.21538

Wang, J., Shi, L., Zhai, L., Zhang, H., Wang, S., Zou, J., Shen, Z., Lian, C., Chen, Y., 2021. Analysis of the long-term effectiveness of biochar immobilization remediation on heavy metal contaminated soil and the potential environmental factors weakening the remediation effect: A review. Ecotoxicol. Environ. Saf. 207, 111261. https://doi.org/10.1016/j.ecoenv.2020.111261

Wang, L., Xiao, H., He, N., Sun, D., Duan, S., 2019. Biosorption and Biodegradation of the Environmental Hormone Nonylphenol By Four Marine Microalgae. Sci. Rep. 9, 5277. https://doi.org/10.1038/s41598-019-41808-8

Xiong, J.-Q., Kurade, M.B., Jeon, B.-H., 2017a. Biodegradation of levofloxacin by an acclimated freshwater microalga, Chlorella vulgaris. Chem. Eng. J. 313, 1251–1257. https://doi.org/10.1016/j.cej.2016.11.017

Xiong, J.-Q., Kurade, M.B., Kim, J.R., Roh, H.-S., Jeon, B.-H., 2017b. Ciprofloxacin toxicity and its co-metabolic removal by a freshwater microalga Chlamydomonas mexicana. J. Hazard. Mater. 323, 212–219. https://doi.org/10.1016/j.jhazmat.2016.04.073

Xu, G., Zhao, X., Zhao, S., He, J., 2021. Acceleration of polychlorinated biphenyls remediation in soil via sewage sludge amendment. J. Hazard. Mater. 420, 126630. https://doi.org/10.1016/j.jhazmat.2021.126630

Yang, J., Gou, Y., Fang, F., Guo, J., Lu, L., Zhou, Y., Ma, H., 2018. Potential of wastewater treatment using a concentrated and suspended algal-bacterial consortium in a photo membrane bioreactor. Chem. Eng. J. 335, 154–160. https://doi.org/10.1016/j.cej.2017.10.149

Yang, X., Zheng, X., Wu, L., Cao, X., Li, Y., Niu, J., Meng, F., 2018. Interactions between algal (AOM) and natural organic matter (NOM): Impacts on their photodegradation in surface waters. Environ. Pollut. 242, 1185–1197. https://doi.org/10.1016/j.envpol.2018.07.099

Zhang, H., Yuan, X., Xiong, T., Wang, H., Jiang, L., 2020. Bioremediation of co-contaminated soil with heavy metals and pesticides: Influence factors, mechanisms and evaluation methods. Chem. Eng. J. 398, 125657. https://doi.org/10.1016/j.cej.2020.125657

Zhang, L., Shen, Z., Fang, W., Gao, G., 2019. Composition of bacterial communities in municipal wastewater treatment plant. Sci. Total Environ. 689, 1181–1191. https://doi.org/10.1016/j.scitotenv.2019.06.432

Zhang, M., Ming, Y., Guo, H., Zhu, Y., Yang, Y., Chen, S., He, L., Ao, X., Liu, A., Zhou, K., Zou, L., Liu, S., 2021. Screening of lactic acid bacteria for their capacity to bind

cypermethrin in vitro and the binding characteristics and its application. Food Chem. 347, 129000. https://doi.org/10.1016/j.foodchem.2021.129000

Zhao, R., Ma, X., Xu, J., Zhang, Q., 2018. Removal of the pesticide imidacloprid from aqueous solution by biochar derived from peanut shell. BioRes 13, 5656–5669.

Zhao, S.-Y., Chen, C.-X., Ding, J., Yang, S.-S., Zang, Y.-N., Qin, X.-D., Gao, X.-L., Song, Z., Ren, N.-Q., 2021. Fabrication of AQ2S/GR composite photosensitizer for the simulated solar light-driven degradation of sulfapyridine. Environ. Sci. Ecotechnology 8, 100111. https://doi.org/10.1016/j.ese.2021.100111