water

MDPI

Article

Contaminant Removal from Wastewater by Microalgal Photobioreactors and Modeling by Artificial Neural Network

Amin Mojiri [1], Noriatsu Ozaki [1], Reza Andasht Kazeroon [2], Shahabaldin Rezania [3], Maedeh Baharlooeian [4], Mohammadtaghi Vakili [5], Hossein Farraji [6], Akiyoshi Ohashi [1], Tomonori Kindaichi [1] and John L. Zhou [7,*]

1 Department of Civil and Environmental Engineering, Graduate School of Advanced Science and Engineering, Hiroshima University, Higashihiroshima 739-8527, Japan
2 Faculty of Civil Engineering, University Technology Mara (UiTM), Shah Alam 40450, Malaysia
3 Department of Environment and Energy, Sejong University, Seoul 05006, Republic of Korea
4 Department of Marine Biology, Faculty of Marine Science and Oceanography, Khorramshahr University of Marine Science and Technology, Khorramshahr 64199-34619, Iran
5 Green Intelligence Environmental School, Yangtze Normal University, Chongqing 408100, China
6 School of Physical and Chemical Sciences, University of Canterbury, Christchurch 8140, New Zealand
7 Centre for Green Technology, School of Civil and Environmental Engineering, University of Technology Sydney, 15 Broadway, Sydney, NSW 2007, Australia
* Correspondence: junliang.zhou@uts.edu.au

Abstract: The potential of microalgal photobioreactors in removing total ammonia nitrogen (TAN), chemical oxygen demand (COD), caffeine (CAF), and *N*,*N*-diethyl-m-toluamide (DEET) from synthetic wastewater was studied. *Chlorella vulgaris* achieved maximum removal of 62.2% TAN, 52.8% COD, 62.7% CAF, and 51.8% DEET. By mixing *C. vulgaris* with activated sludge, the photobioreactor showed better performance, removing 82.3% TAN, 67.7% COD, 85.7% CAF, and 73.3% DEET. *Proteobacteria*, *Bacteroidetes*, and *Chloroflexi* were identified as the dominant phyla in the activated sludge. The processes were then optimized by the artificial neural network (ANN). High R^2 values (>0.99) and low mean squared errors demonstrated that ANN could optimize the reactors' performance. The toxicity testing showed that high concentrations of contaminants (>10 mg/L) and long contact time (>48 h) reduced the chlorophyll and protein contents in microalgae. Overall, a green technology for wastewater treatment using microalgae and bacteria consortium has demonstrated its high potentials in sustainable management of water resources.

Keywords: bacteria; caffeine; DEET; emerging contaminants; microalgae

Citation: Mojiri, A.; Ozaki, N.; Kazeroon, R.A.; Rezania, S.; Baharlooeian, M.; Vakili, M.; Farraji, H.; Ohashi, A.; Kindaichi, T.; Zhou, J.L. Contaminant Removal from Wastewater by Microalgal Photobioreactors and Modeling by Artificial Neural Network. *Water* **2022**, *14*, 4046. https://doi.org/10.3390/w14244046

Academic Editor: Alejandro Gonzalez-Martinez

Received: 23 November 2022
Accepted: 8 December 2022
Published: 12 December 2022

1. Introduction

Water resources supply needs for drinking, industrial and agricultural activities, and habitat for diverse wildlife [1]. The discharge of harmful contaminants into aquatic environments is a major concern that can pose serious threats to ecosystems and human health [2]. Currently, emerging pollutants (i.e., pharmaceuticals and personal care products, PPCPs) have gained global attention [3] because they have been extensively reported to occur in numerous water bodies worldwide, and constitute a serious threat to human health [4]. PPCPs are commonly applied in the cosmetic industry, medicine, livestock farming, aquaculture, and agriculture; as they cannot be effectively removed in wastewater treatment plants, they have been detected in aquatic environments worldwide [5]. Although PPCPs usually occur at trace concentrations in aquatic environments, most of these compounds can cause endocrine disruption, chronic toxicity in humans and organisms, and increased antibacterial resistance [6].

Contrary to the prescription PPCPs, caffeine (CAF) and *N*,*N*-diethyl-*m*-toluamide (DEET) are commonly available for commercial, household, and industrial uses. DEET is applied in several commercial products of insect repellent to protect animals and humans

against insects and mosquitoes [7]. Tran et al. [7] reported that wastewater, groundwater, and surface water samples all contained DEET. CAF is an alkaloid that belongs to the methylxanthine family, which can be found in teas, coffees, sodas, and chocolate products. CAF is commonly consumed due to its stimulating effect on the central nervous system [4], with the average daily consumption of 70–400 mg per person worldwide [8].

As conventional wastewater treatment plants are not efficient in eliminating PPCPs [9], new technologies to remove PPCPs are needed. Physical and chemical methods (i.e., adsorption) have been employed to eliminate PPCPs, but these methods produce secondary contaminations [10]. Thus, the application of biological techniques (e.g., microalgae-based systems) can be considered as a green method to remove contaminants from water bodies [11]. Microalgae grow quickly, treat wastewater, and generate biomass for future use (such as production of biofuel and biochar). Moreover, microalgae consume CO_2, absorb sunlight, and produce O_2, making the microalgal method an environmentally friendly system for treating wastewater [6]. Marchão et al. [12] stated that using microalgae to remove pollutants from water bodies is an effective and economic approach because they can grow in different wastewater streams, efficiently recycle nutrients, require low energy, and reduce the formation of waste sludge. Amongst different microalgae species, *Chlorella vulgaris* is a great candidate for wastewater treatment owing to its strong ability to consume nutrients, remove chemical oxygen demand (COD), and reduce emerging contaminants [13]. Hence, *C. vulgaris* was selected in this study. It belongs to the *Chlorellaceae* family and is one of the most notable green eukaryotic microalgae [14]. To improve the efficiency of the algae in removing emerging contaminants, researchers have suggested coculture or a consortium of microorganisms [15]. For instance, Rossi et al. [16] stated that the cocultivation of microalgae–bacteria consortia seems to have higher resistance toward free ammonia and metal ions than algal monocultures. In addition to removing a portion of the emerging contaminants, microalgae enhance the biodegradation of these contaminants by bacteria, because the microalgae supply oxygen (as an electron acceptor) via photosynthesis for the aerobic bacteria [17]. The 96% removal of 4-nonylphenol with a microbial consortium including *C. vulgaris* has been reported [14].

It is stated that activated sludge has a vital role in the protection of the environment and human health because activated sludge contains a complex and diverse microbial community that contributes considerably to the degradation of organic matter, abatement of nutrients, and detoxification of water and wastewater [16]. Therefore, activated sludge was used as a part of the consortium in this study. During the treatment of water and wastewater with biological methods, an artificial neural network (ANN) has been employed to control, monitor, and simulate the treatment processes [18].

This study thus aimed to compare the removal effectiveness of the target contaminants by microalgae and microalgae/activated sludge consortium, optimize the process performance for the contaminants removal by using an ANN, and determine the ecotoxicity of PPCPs on the chlorophyll and protein content of microalgae.

2. Materials and Methods

2.1. Materials and Proparation of Synthetic Wastewater

Chlorella vulgaris was collected from a stirred-tank photobioreactor with a hydraulic retention time (HRT) of 7 days at Amin-Azma Research Institute laboratory, which was operated at a temperature of 25 ± 2 °C and an illumination intensity of 85–110 µmol photons/(m^2 s). The cultivation of *Chlorella vulgaris* was done in a 500 mL Erlenmeyer flask containing BG11 medium. The activated sludge biomass was collected from a sequencing batch reactor (SBR) operating in the laboratory. The SBR (working value of 3.5 L) included filling (20 min), reacting (1320 min), settling (85 min), drawing, and idle (15 min) phases, which treated domestic wastewater. DEET ($C_{12}H_{17}NO$, CAS number: 134-62-3) and CAF ($C_8H_{10}N_4O_2$, CAS number: 58-08-2) with purities of >97% were obtained from Sigma-Aldrich Co. (Petaling Jaya, Malaysia). For preparation of stock solutions (1 g/L), these PPCPs were dissolved separately in distilled water.

Tap water was used to prepare synthetic wastewater. The tap water was characterized for pH (7.09), hardness (46.1 mg/L), Ca^{2+} (9.1 mg/L), K^{+} (0.7 mg/L), Cl^{-} (3.7 mg/L), NH_4^{+} (0.01 mg/L), Na^{+} (0.9 mg/L), Mg^{2+} (0.6 mg/L), and electrical conductivity (EC, 169 µS/cm). The synthetic wastewater contained nutrients and trace elements as described by Makut et al. [19], and supplemented with TAN, COD, CAF, and DEET. To reach the concentration of 50–200 mg/L of TAN, ammonium chloride (NH_4Cl) was added to the tap water. In addition, carbon sources (including yeast, milk powder, and peptone) were added to react with the COD (50–200 mg/L). CAF and DEET (0.20–2.2 mg/L) were added to the synthetic wastewater by diluting their stock solutions.

2.2. Photobioreactors

The removal of contaminants COD, TAN, CAF, and DEET by the consortium of microalgae and activated sludge was studied in two identical laboratory-based photobioreactors. The photobioreactors (6.0 L) were made of glass that were 16 cm in diameter and 30 cm in depth. They were operated under light (12 h):dark (12 h) cycles at an illumination intensity of 100 ± 15 µmol photons/m^2 s, at 25 ± 2 °C. Reactor 1 was inoculated with both *C. vulgaris* (1 g/L) and activated sludge biomass (1 g/L) based on the preliminary experiments, which was in agreement with the study of Yang et al. [20]. Reactor 2 was inoculated with 1 g/L of *C. vulgaris* only. The performance of reactor 1 (microbial consortium) and reactor 2 (microalgae) could then be compared. The synthetic wastewater was treated with both reactors to monitor their performance. The aeration rate was 0.4 L/min, and the pH of the influent was adjusted to 7.0 [4]. The system operating conditions are shown in Table 1. All experiments were conducted in triplicate, and the average and standard deviations values were calculated.

Table 1. Operation conditions used in the different experiments.

Experiment	Contact Time (d)	Initial Concentration of PPCPs (mg/L)	Initial Concentration of TAN and COD (mg/L)
1	1–7	0.2	50
2	1–7	0.6	80
3	1–7	1.0	110
4	1–7	1.4	140
5	1–7	1.8	170
6	1–7	2.2	200

2.3. DNA Extraction and Microbial Community in Activated Sludge

Before inoculation of the photobioreactor and after running the experiments, the community of bacteria in the activated sludge was studied. The extraction of DNA was carried out in triplicate with the E.Z.N.A.® Soil DNA Kit, based on the manufacturer's instructions (Omega Biotek, Norcross, GA, USA). The V3–V4 regions of the 16S rRNA genes were amplified by the sets of primer 341F and 805R [21]. The mixture of PCR (30 µL) contained 15 µL of 2 × Taq master Mix, 1 µL of Bar-PCR primer F (10 µM), Genomic DNA 10–20 ng, and 1 µL of Primer R (10 µM). The PCR process began at 94 °C for 3 min; then with 5 cycles at 94 °C for 30 s, 20 s at 45 °C, 30 s at 65 °C; followed by 20 cycles at 94 °C for 20 s, 20 s at 55 °C, at 30 s for 72 °C; and at 72 °C for 5 min [22]. Then, the DNA purified in the study was sequenced by the MiSeq platform using a MiSeq Reagent Kit v3 (Illumina Inc., San Diego, CA, USA), and taxonomic units (OTUs) were studied. The claustration of sequences into OTUs was done by the ARB software (6.0) [23].

2.4. Photolysis of Contaminants

During contaminant removal in the two photobioreactors, there is a potential that the contaminants may be removed by direct photolysis. Hence, the photolysis of contaminants was determined in a photolysis reactor (Erlenmeyer flasks), which was capped during the photolysis process [4]. The flasks (without the presence of microalgae or activated sludge) comprised the solution of CAF and DEET (0.2–2.2 mg/L) under illumination

intensity of 100 ± 15 µmol photons m^{-2} s^{-1}. Similar to the photobioreactors, the photolysis experiments were run under light:dark cycles of 12:12 h. By measuring the concentrations of CAF and DEET in the solution, the extent of PPCPs removal by the photolysis process was determined.

2.5. Analyses of Contaminant Concentrations in Water

A high-pressure liquid chromatograph (LC-20AT, Shimadzu Co., Ltd., Tokyo, Japan) with a UV detector (wavelength of 273 nm and 250 nm) and a C_{18} analytical column was employed to measure the concentrations of emerging contaminants. At 1.1 mL/min flow rate, the mobile phase comprised methanol and ultrapure water (70/30, v/v). The detection limit of contaminants analysis was expressed as three times the standard deviation of the baseline noise [4]. A Hach DR 2800 (Hach, Loveland, CO, USA) was used to analyze COD (Method 8000) and TAN (Method 8038, Nessler Method) in water samples.

2.6. Optimization of Photobioreactors by Artificial Neural Network

Based on the measurement of contaminants concentrations, the abatement efficiency of COD, TAN, DEET, and CAF was calculated by Equation (1):

$$\text{Removal efficiency } (\%) = \frac{\textit{Initial concentration } \left(\frac{\text{mg}}{\text{L}}\right) - \textit{Final concentration } \left(\frac{\text{mg}}{\text{L}}\right)}{\textit{Initial concentration } \left(\frac{\text{mg}}{\text{L}}\right)} \times 100 \quad (1)$$

To improve the performance (Tables S1 and S2) of photobioreactors, an ANN, which is a mathematical computational approach based on the human nervous system, was used. An ANN has a great capability to estimate and predict complex nonlinear processes [24]. In this study, the ANN approach was established by using the nftool function in MATLAB R2015a to optimize the relationship between two independent factors, the initial concentrations of PPCPs (0.2–2.2 mg/L) and contact time (1–7 d), and the response (i.e., removal of PPCPs). The whole dataset was divided into training (60%), validation (20%), and testing (20%). Multilayer feedforward ANNs were employed. The Levenberg–Marquardt (LM) method was applied to train the ANN model. At the maximum validation, failures are equal to zero and the validation is disabled. Model performance was assessed by the values of correlation coefficient (R^2) and mean square error (MSE), as shown in Equations (2) and (3) [4]:

$$R^2 = 1 - \frac{\sum_{i=1}^{N}\left(\left|y_{prd,i} - y_{exp,i}\right|\right)}{\sum_{i=1}^{N}\left(\left|y_{prd,i} - y_m\right|\right.} \quad (2)$$

$$MSE = \frac{1}{N}\sum_{i=1}^{N}\left(\left|y_{prd,i} - y_{exp,i}\right|\right)^2 \quad (3)$$

where the anticipated value of the ANN is specified by $y_{prd,i}$, and $y_{exp,i}$ is the experimental value, N is the number of data points, and the average value of experiments is represented by y_m.

2.7. Effects of CAF and DEET on Chlorella vulgaris

The potential adverse effects of contaminants CAF and DEET on microalgae were studied. The Erlenmeyer flasks were added with *Chlorella vulgaris* (5×10^4 cells/mL) which were mixed with different concentrations of CAF and DEET (0–35 mg/L) for different contact time (0–72 h). The flasks were run at the illumination intensity of 100 ± 15 µmol photons/m^2 s at room temperature, and the pH of the influent was set at 7. The contents of protein and chlorophyll were determined using a UV–Visible spectrophotometer (UV-1280, Shimadzu, Tokyo, Japan). The preparation of samples for tests was completed by collecting 10 mL of culture which was centrifuged at 4500–5000× g rpm for 15 min. The supernatant was discarded, and the container was re-suspended in 10 mL of methanol (>90%), incubated at 60 °C for 10–15 min, and then centrifuged again for 10–15 min [25].

For testing chlorophyll, the samples were measured at wavelengths of 646 nm and 663 nm. Chlorophyll (Chl) contents were then estimated by Equations (4)–(6) [26]:

$$Chl\ a\ (\mu g/mL) = 12.25\ (A_{663}) - 2.55\ (A_{646}) \quad (4)$$

$$Chl\ b\ (\mu g/mL) = 20.13\ (A_{646}) - 4.91\ 9(A_{663}) \quad (5)$$

$$Chl\ a + b\ (\mu g/mL) = 17.76\ (A_{646}) + 7.34\ (A_{663}) \quad (6)$$

where A_{646} and A_{663} represent the absorbance values at the wavelengths of 646 nm and 663 nm, respectively.

For testing the content of protein, the samples were scanned at wavelengths 260 nm and 280 nm. The content of protein was estimated by Equation (7) [27]:

$$Protein\ content = (1.55 \times A_{280}) - (0.77 \times A_{260}) \quad (7)$$

where A_{260} and A_{280} indicate the absorbance values at the wavelengths of 260 nm and 280 nm, respectively.

3. Results

3.1. Photodegradation of PPCPs

Most PPCPs can be removed by more than one pathway, such as biodegradation and photodegradation; thus, the removal of CAF and DEET via the photolysis process was tested. Molecules of emerging contaminants can absorb light, leading to breaking bonds, in direct photodegradation [28]. Based on this study, the direct photolysis process eliminated 13% (contact time of 1 d and initial concentration of 2.2 mg/L of PPCPs) to 19% (contact time of 7 d and initial concentration of 0.6 mg/L of PPCPs). In comparison with the removal performance of the reactor in total (which is discussed in the next section), the findings indicate that the main pathway to remove emerging contaminants was biodegradation, consistent with de Wilt et al. [29].

3.2. Contaminant Removal in Photobioreactors

The results from this study included the removal of TAN, COD, CAF, and DEET by two photobioreactors, reactor 1 comprising microalgae and activated sludge and reactor 2 comprising microalgae only. The performance of both reactors is compared in Figure 1. Moreover, the biomass evolution in both reactors during the treatment process is shown in Figure 2. Then, an ANN was employed to optimize the removal performance of both reactors in the elimination of PPCPs. Finally, the toxic effects of PPCPs on microalgae were investigated.

As indicated in Figure 1, for the first photobioreactor, the maximum elimination of COD and TAN was 82.3% and 67.7%, respectively, when the reaction time was 5 d, and the initial concentration of TAN and COD was 80 mg/L. Furthermore, for the first photobioreactor, the optimum abatement of CAF and DEET was 85.7% and 73.3%, respectively, when the contact time was 5 d, and the initial concentration of PPCPs was 1.0 mg/L, while the minimum removal of CAF and DEET was 52.6% and 41.8% at a contact time of 1 d and with an initial concentration of 2.2 mg/L.

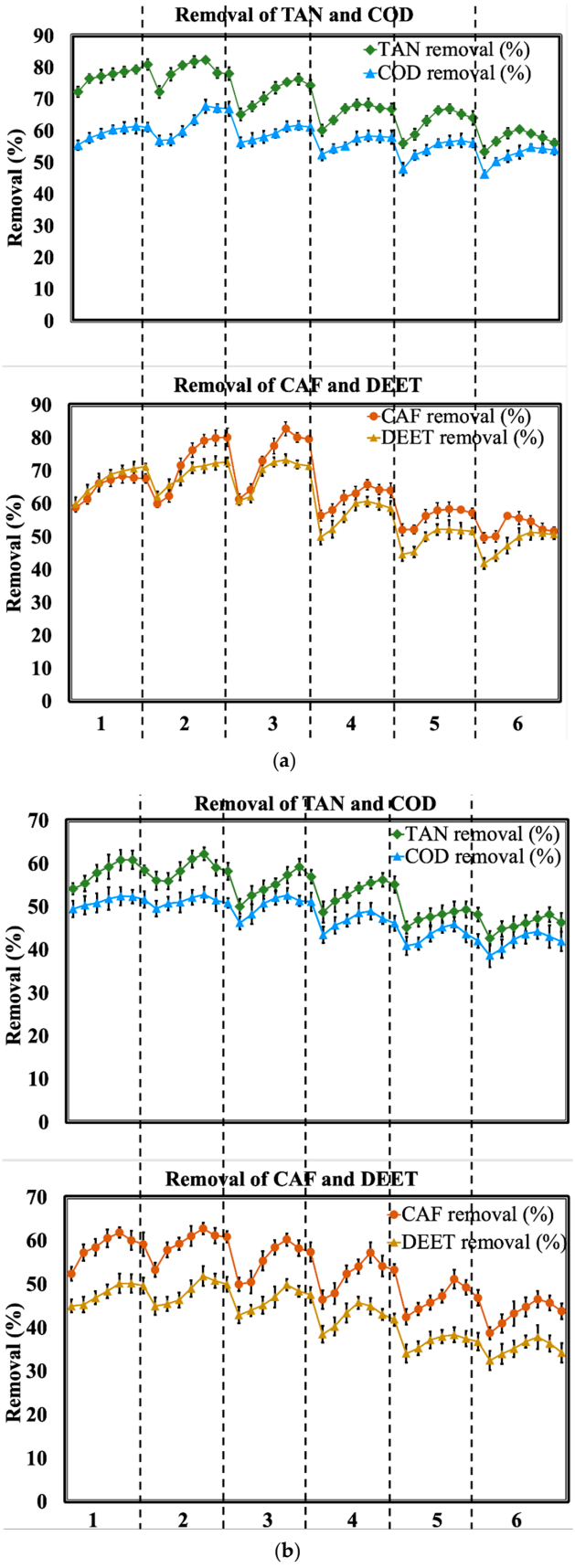


Figure 1. The performance of (**a**) reactor 1 and (**b**) reactor 2 in removing TAN, COD, CAF, and DEET.

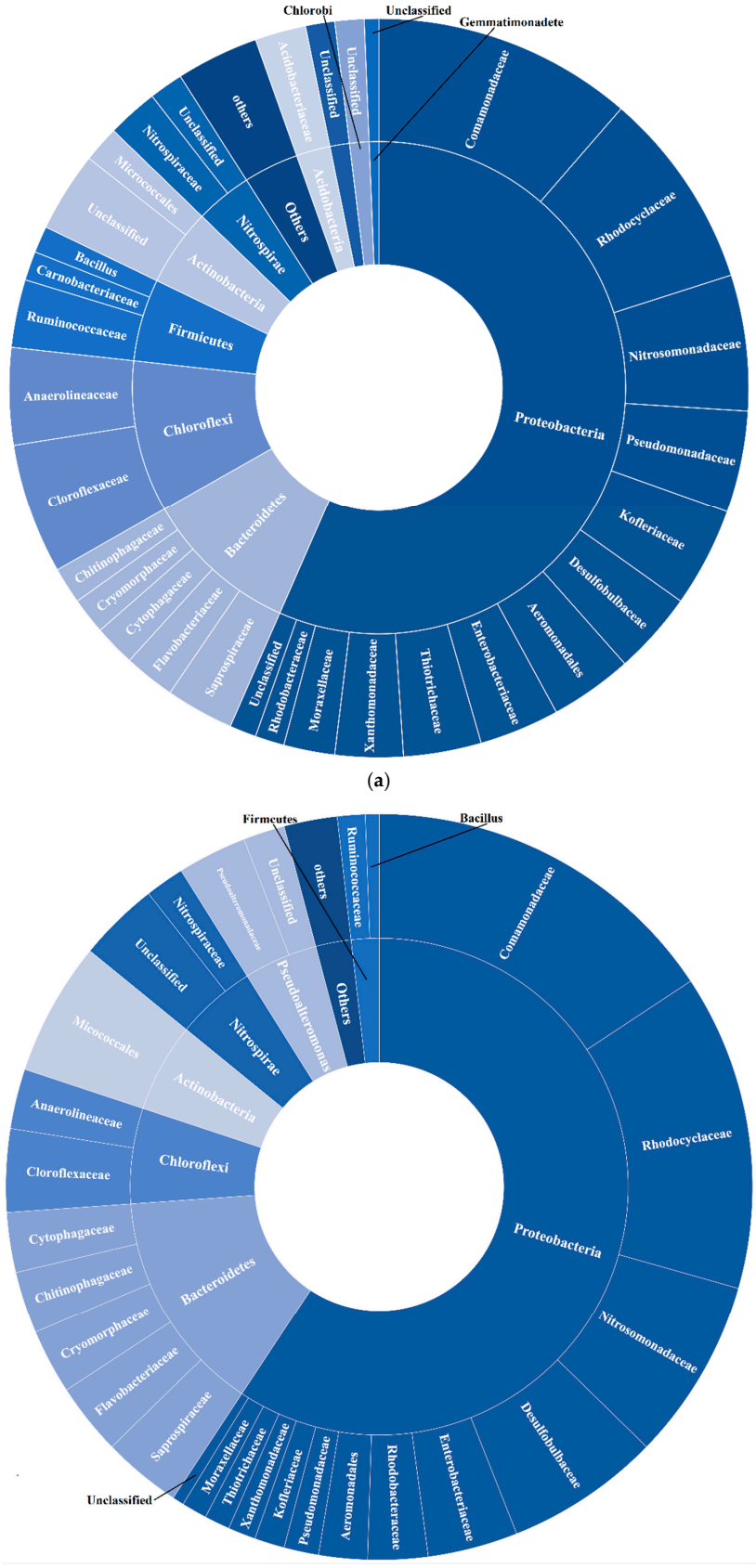


Figure 2. The microbial community in activated sludge before (**a**) and after (**b**) the operation in reactor 1.

For the second reactor, Figure 1 shows that the maximum abatement of 62.2% TAN and 52.8% COD was achieved at a contact time of 5 d and a concentration of 80.0 mg/L of TAN and COD. Moreover, the maximum elimination of CAF and DEET was 62.7% and 51.8%, respectively, on the fifth day with the initial PPCPs concentration of 0.6 mg/L, while the minimum elimination of CAF and DEET was 38.7% and 32.4%, respectively, during a contact time of 1 d with an initial concentration of 2.2 mg/L of PPCPs. Therefore, the removal of PPCPs by the consortium of microalgae and activated sludge in reactor 1 was more than that in the second reactor. Microalgae can improve the degradation of emerging contaminants by bacteria not only by releasing oxygen for aerobic bacteria but also by releasing dissolved organic matter [17]. Presentato et al. [30] stated that an alternative carbon substrate is required to achieve biotic transformation of organic contaminants in bacteria, which is supplied by microalgae in this case. García-Galán et al. [31] reported 50% removal of carbamazepine using a semi-closed microalgae photobioreactor. In another study, 12% of carbamazepine was eliminated by *C. vulgaris* [32], which is in line with the second reactor in the study. Meng et al. [33] expressed that some bacteria (such as *Proteobacteria* and *Bacteroidetes*) could eliminate the emerging contaminants. Apart from that, Matamoros et al. [34] stated that the microalgae were able to remove emerging contaminants. Thus, the performance of reactor 1 was improved in comparison with the second photobioreactor because of the consortium of microalgae and bacteria used. da Silva Ribeiro et al. [35] reported that >54% of sulfamethoxazole was removed by the microalgae/bacteria consortium in less than 1 week, which is in line with the current study. Mojiri et al. [4] reported a 39.8% removal of sulfamethoxazole during 6.3 days, which is in agreement with the findings of the second reactor in this study. In addition, the first reactor showed better performance in the removal of TAN and COD. In one study, 84.5% of ammonia and 71.8% of COD were removed in a modified treatment method including *Chlorella sorokiniana* [36], which is consistent with the current study. Similarly, Akizuki et al. [37] eliminated more than 80% of ammonia by the microalgae/bacteria consortium. Wang et al. [38] and Rossi et al. [16] stated that the high concentration of ammonia can inhibit the growth and activity of microalgae because ammonia enters into the cell interior by directly crossing the cell membrane, causing toxic effects on the enzymes related to the photosynthesis process. However, the high concentration of ammonia did not cause significant effects on the algae in the first reactor, and the removal performance was stable in most runs due to the presence of bacteria. *Proteobacteria*, *Bacteroidetes*, *Nitrospirae*, and *Chloroflexi* have a vital role in removing high concentrations of ammonia [39], which were the dominant bacteria in the first reactor during the study. The COD removal in reactor 1 was high because photosynthesis of microalgae generates oxygen, which is necessary for the bacteria to metabolize organic matter. Thus, the COD removal would be higher in a consortium of microalgae and bacteria [40]. Zhu et al. [41] stated that *C. curvatus* could consume some fraction of COD.

3.3. Evolution of Microbial Biomass

Based on Figure 2a, the dominant bacteria were *Proteobacteria*, *Bacteroidetes*, and *Chloroflexi* in the activated sludge, which are the same as with a study reported by Zhang et al. [42]. In the co-culture of activated sludge and microalgae, after running the experiments, the microbial communities can be changed [43]; therefore, the microbial community was monitored in reactor 1 after experiments. Based on Figure 2b, the *Protobacteria*, *Bacteroidetes*, and *Chloroflexi* were still dominant phyla after experiments; however, the percentage of *Protobacteria* was increased, while the percentage of *Bacteroidetes* and *Chloroflexi* was decreased. In comparison with the activated sludge (Figure 2a), the *Actinobacteria* community was significantly increased, which is in line with findings by [44]. This may relate to the light conditions. Maresca et al. [45] stated that *Actinobacteria* grew faster in the presence of light, because the transport of sugar and metabolism were upregulated. Moreover, *Comamonadaceae*, *Desulfobulbaceae*, and *Rhodobacteraceae* were increased after experiments due to better aerobic conditions and the supply of oxygen by microalgae.

As shown in Figure 3, the biomass concentration in reactor 1 was much greater than in reactor 2, which is consistent with the findings of Berthold et al. [46].

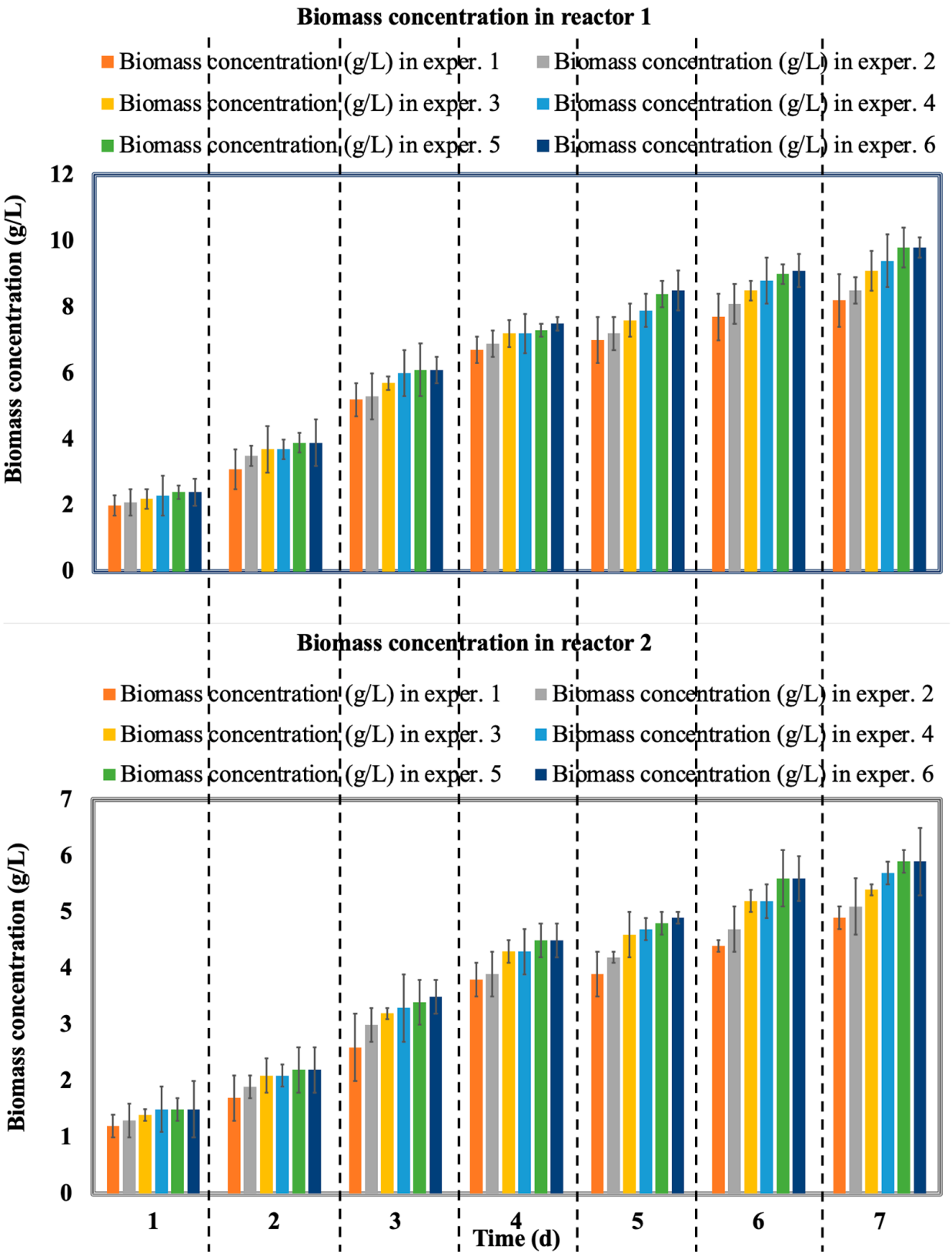


Figure 3. The evolution of biomass concentration (mg dry weight/L) in reactors 1 and 2 over time.

In reactor 1, the maximum concentration of biomass was 9.8 g/L, compared with the maximum biomass concentration of 5.9 g/L in reactor 2. Moreover, the biomass concentration increased with an increase in the concentrations of contaminants and TAN. Mojiri et al. [4] suggested that low concentrations of some emerging contaminants have a positive effect on chlorophyll accumulation and the growth of microalgae. When compared to microalgae only, Makut et al. [19] reported more than a 21% enhancement of biomass in a bacteria/microalgae consortium. In a microalgae/activated sludge consortium (1:1), the concentration of biomass increased from 0.5 mg/L to more than 0.95 mg/L (89.8% biomass productivity) during 48 h, which is in line with the current study. Ray et al. [47] expressed that enhanced biomass production through the coculture strategy helped the bioremediation process as well as efficient biomass harvesting. Microalgae/bacterial symbiosis offers several advantages in comparison with algal monocultures: algae provide oxygen with photosynthesis, different organic exudates improve the growth of bacteria, and algae secrete several toxic metabolites that inhibit undesired bacterial growth, thus preventing competition among different bacteria in the coculture system [47] and reducing the effects of contaminates on microalgae. In addition, the biomass increased with time, which is in line with other findings [48].

3.4. Optimization of Removal Performance by the ANN

Once the contaminants were being removed by the two reactors, the removal optimization of both reactors was conducted by the ANN. Two neurons in the input layer were contact time (h) and the initial concentration of PPCPs (mg/L), while there were five neurons in the hidden layer, and removal (%) of CAF and DEET was the output layer. For developing a model via an ANN, training is the most vital step; therefore, 60% of data were clustered in the training step. The analysis of linear regression between the anticipated and experimental abatement data for CAF and DEET was conducted to evaluate the network efficiency [35]. For the removal of CAF and DEET by the first reactor, the experimental values versus the model prediction for the optimum topology for training, validation, and test data are presented in Figure 4. A high R^2 value (0.99 for CAF and DEET) denotes the reliability of the model. Kassahun et al. [2] stated that the high R^2 value can confirm that the ANN topology exhibits a good estimate and possesses an appropriate generalization capability in order to predict the degradation efficiency under different operating conditions. The values of MSE were obtained by the LM training algorithm after 6 and 8 epochs (Figure S1) for the removal of CAF and DEET, respectively.

For the removal of CAF and DEET by the second reactor, the experimental values versus the model prediction for the optimum topology for testing, validation, and training data are indicated in Figure 5. A high R^2 value (0.99) for both the removal of CAF and DEET denotes the reliability of the model. The values of MSE were obtained by the LM after 7 and 13 epochs (Figure S2) for the removal of CAF and DEET, respectively.

3.5. Effects of Emerging Contaminants on Microalgae

The combined impact of CAF and DEET on *Chlorella vulgaris* has not been widely reported in previous studies. Thus, water polluted with both CAF and DEET (0–35 mg/L) was used in this study. As indicated in Figure 6, the content of chlorophyll and protein increased with increasing PPCPs concentration, up to 5.0–10.0 mg/L. Then, the protein and chlorophyll were dramatically decreased by further increasing exposure time (contact time) and PPCP concentration. Low concentrations of emerging contaminants increased chlorophyll and protein content, due to the increase in enzyme synthesis or other energy-producing fractions [4]. However, a high concentration of PPCPs can negatively affect photosynthesis of *Chlorella pyrenoidosa* [49]. Moreover, the longer period of exposure to emerging contaminants negatively influenced the growth and cellular response of *Chlorella* sp. [49]. In addition, a high concentration of emerging contaminants may result in a decrease in the assimilation of CO_2 by affecting the photosynthetic carbon-reduction-cycle enzymes [48].

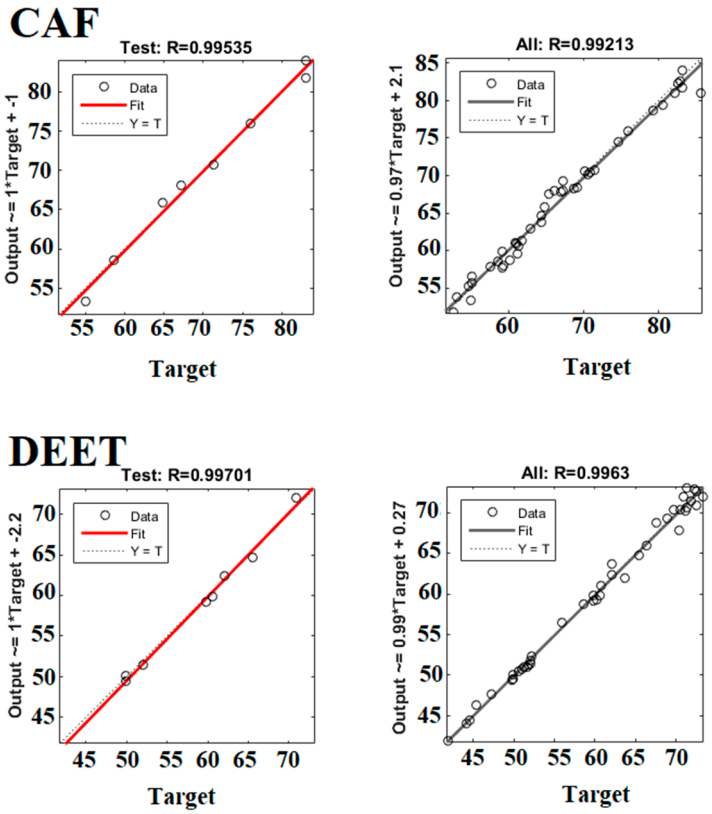


Figure 4. Prediction versus experimental values for the optimum analysis of the test and all data for the elimination of CAF and DEET in reactor 1.

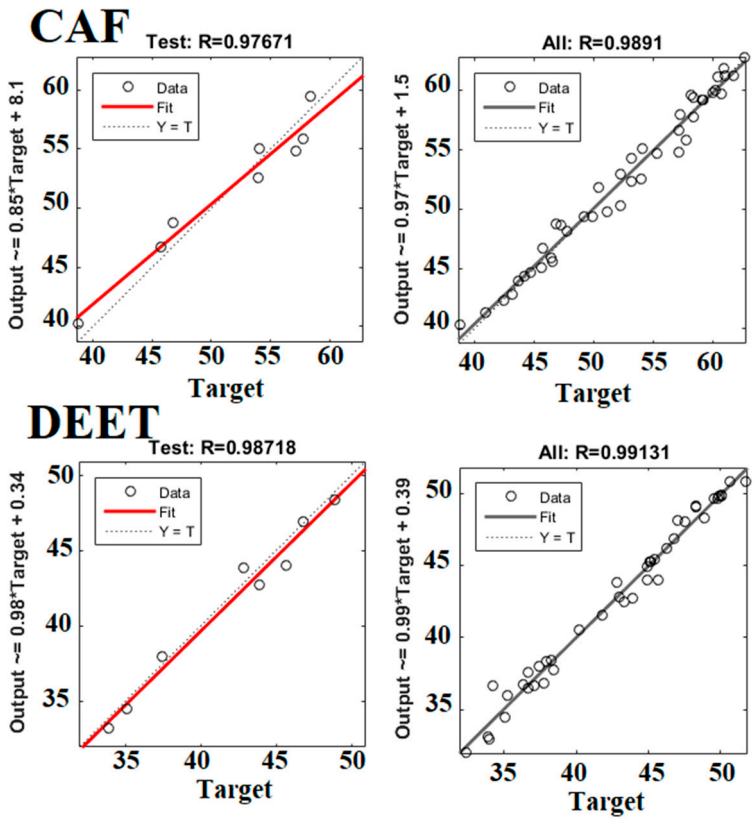
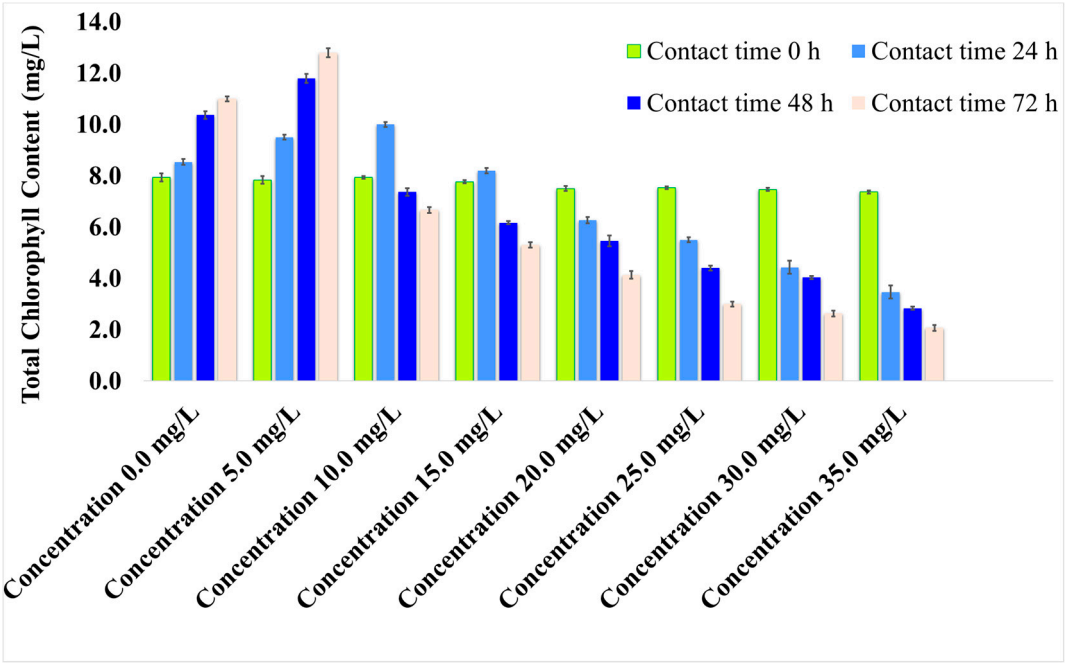


Figure 5. Prediction and experimental values for the optimum analysis of the test and all data for the elimination of CAF and DEET in reactor 2.

(**a**)



(**b**)

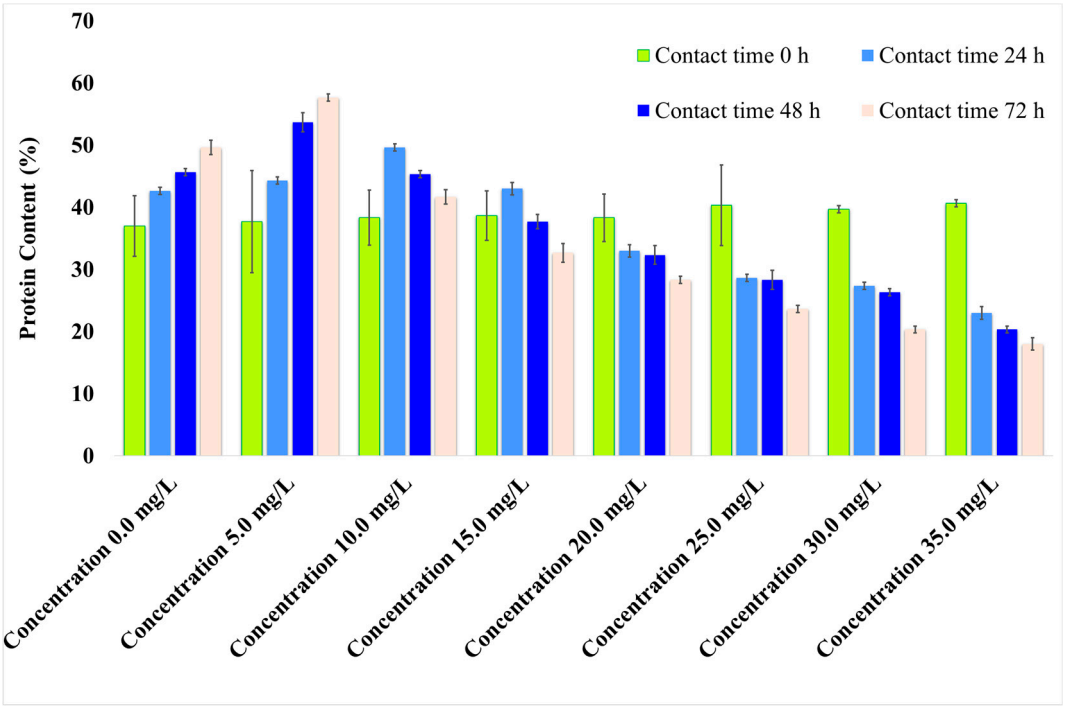


Figure 6. The effects of CAF and DEET on (**a**) chlorophyl and (**b**) protein content in microalgae.

4. Conclusions

The removal of TAN, COD, CAF, and DEET from synthetic wastewater was investigated in two photobioreactors, including the first reactor (integrated *Chlorella vulgaris*/activated sludge) and the second reactor (*Chlorella vulgaris*). The findings of the study are summarized below:

1. The biological consortium in photobioreactor 1 removed a maximum of 82.3% TAN and 67.7% COD, higher than 62.2% TAN and 52.8% COD by microalgae alone.
2. The consortium achieved a maximum removal of 85.7% for CAF and 73.3% for DEET, which was higher than 62.7% and 51.8% by microalgae alone.

3. ANN was able to optimize photobioreactor performance, as demonstrated by the high R^2 (>0.99) and low MSE (<0.1) values.
4. High concentrations of PPCPs and long contact time reduced the content of chlorophyll and protein in microalgae.

Further investigations are needed as follows: (1) The tolerance of the photobioreactors and their stable operation under the stress of multiple contaminants should be further assessed. (2) Separately, the cocktail effects of different abiotic factors and contaminants can affect the performance of microalgae, which needs further study.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w14244046/s1, Table S1: Performance of reactor 1 in the removal of pollutants; Table S2: Performance of reactor 2 in the removal of pollutants; Figure S1: MSE versus the number of epochs for the removal of CAF (A) and DEET (B) in reactor 1; Figure S2: MSE versus the number of epochs for the removal of CAF (A) and DEET (B) in reactor 2.

Author Contributions: Conceptualization, A.M.; methodology, A.M., M.B. and H.F.; software, A.M. and R.A.K.; validation, M.B., H.F., A.M. and S.R.; investigation, M.B. and H.F.; resources, H.F.; data curation, A.M.; writing—original draft preparation, A.M. and J.L.Z.; writing—review and editing, A.M. and J.L.Z.; visualization, J.L.Z. and M.V.; supervision, A.M.; project administration, A.M.; N.O., A.O. and T.K. All authors have read and agreed to the published version of the manuscript.

Funding: This study was funded by the JSPS KAKENHI (grant number 21H01465).

Data Availability Statement: Not applicable.

Acknowledgments: We thank the Amin-Azma Research Institute for providing the facilities to carry out the research.

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

References

1. Kargol, A.K.; Cao, C.; James, C.A.; Gough, H.L. Wastewater reuse for tree irrigation: Influence on rhizosphere microbial communities. *Resour. Environ. Sustain.* **2022**, *9*, 100063. [CrossRef]
2. Kassahun, S.K.; Kiflie, Z.; Kim, H.; Baye, A.F. Process optimization and kinetics analysis for photocatalytic degradation of emerging contaminant using N-doped TiO_2-SiO_2 nanoparticle: Artificial Neural Network and Surface Response Methodology approach. *Environ. Technol. Innov.* **2021**, *23*, 101761. [CrossRef]
3. Zhao, H.; Zhou, J.L.; Zhang, J. Tidal impact on the dynamic behavior of dissolved pharmaceuticals in the Yangtze Estuary, China. *Sci. Total Environ.* **2015**, *536*, 946–954. [CrossRef] [PubMed]
4. Mojiri, A.; Baharlooeian, M.; Zahed, M.A. The Potential of Chaetoceros muelleri in Bioremediation of Antibiotics: Performance and Optimization. *Int. J. Environ. Res. Public Health* **2021**, *18*, 977. [CrossRef] [PubMed]
5. Zhao, J.-R.; Fan, X.-Y.; Li, X.; Gao, Y.-X.; Zhang, Z.-X. Impact of ciprofloxacin and copper combined pollution on activated sludge: Abundant-rare taxa and antibiotic resistance genes. *Bioresour. Technol.* **2022**, *349*, 126882. [CrossRef]
6. Mojiri, A.; Zhou, J.L.; Ratnaweera, H.; Rezania, S.; Nazari, V.M. Pharmaceuticals and personal care products in aquatic environments and their removal by algae-based systems. *Chemosphere* **2022**, *288*, 132580. [CrossRef]
7. Tran, N.H.; Hu, J.; Urase, T. Removal of the insect repellent *N*,*N*-diethyl-m-toluamide (DEET) by laccase-mediated systems. *Bioresour. Technol.* **2013**, *147*, 667–671. [CrossRef]
8. Bachmann, S.A.L.; Calvete, T.; Féris, L.A. Caffeine removal from aqueous media by adsorption: An overview of adsorbents evolution and the kinetic, equilibrium and thermodynamic studies. *Sci. Total Environ.* **2021**, *767*, 144229. [CrossRef]
9. García, J.; García-Galán, M.J.; Day, J.W.; Boopathy, R.; White, J.R.; Wallace, S.; Hunter, R.G. A review of emerging organic contaminants (EOCs), antibiotic resistant bacteria (ARB), and antibiotic resistance genes (ARGs) in the environment: Increasing removal with wetlands and reducing environmental impacts. *Bioresour. Technol.* **2020**, *307*, 123228. [CrossRef]
10. Al Qarni, H.; Collier, P.; O'Keeffe, J.; Akunna, J. Investigating the removal of some pharmaceutical compounds in hospital wastewater treatment plants operating in Saudi Arabia. *Environ. Sci. Pollut. Res.* **2016**, *23*, 13003–13014. [CrossRef]
11. Leng, L.; Li, W.; Chen, J.; Leng, S.; Chen, J.; Wei, L.; Peng, H.; Li, J.; Zhou, W.; Huang, H. Co-culture of fungi-microalgae consortium for wastewater treatment: A review. *Bioresour. Technol.* **2021**, *330*, 125008. [CrossRef] [PubMed]
12. Marchão, L.; Fernandes, J.R.; Sampaio, A.; Peres, J.A.; Tavares, P.B.; Lucas, M.S. Microalgae and immobilized TiO_2/UV-A LEDs as a sustainable alternative for winery wastewater treatment. *Water Res.* **2021**, *203*, 117464. [CrossRef]
13. Terán Hilares, R.; Garcia Bustos, K.A.; Sanchez Vera, F.P.; Colina Andrade, G.J.; Pacheco Tanaka, D.A. Acid precipitation followed by microalgae (*Chlorella vulgaris*) cultivation as a new approach for poultry slaughterhouse wastewater treatment. *Bioresour. Technol.* **2021**, *335*, 125284. [CrossRef] [PubMed]

14. Soleimanikhorramdashti, M.; Samipoorgiri, M.; Majidian, N. Extraction lipids from chlorella vulgaris by supercritical CO_2 for biodiesel production. *S. Afr. J. Chem. Eng.* **2021**, *38*, 121–131. [CrossRef]
15. Romdhane, L.; Panozzo, A.; Radhouane, L.; Dal Cortivo, C.; Barion, G.; Vamerali, T. Root Characteristics and Metal Uptake of Maize (*Zea mays* L.) under Extreme Soil Contamination. *Agronomy* **2021**, *11*, 178. [CrossRef]
16. Rossi, S.; Díez-Montero, R.; Rueda, E.; Castillo Cascino, F.; Parati, K.; García, J.; Ficara, E. Free ammonia inhibition in microalgae and cyanobacteria grown in wastewaters: Photo-respirometric evaluation and modelling. *Bioresour. Technol.* **2020**, *305*, 123046. [CrossRef] [PubMed]
17. Shahsavari, E.; Rouch, D.; Khudur, L.S.; Thomas, D.; Aburto-Medina, A.; Ball, A.S. Challenges and Current Status of the Biological Treatment of PFAS-Contaminated Soils. *Front. Bioeng. Biotechnol.* **2021**, *8*, 602040. [CrossRef]
18. Ebrahimzade, I.; Ebrahimi-Nik, M.; Rohani, A.; Tedesco, S. Towards monitoring biodegradation of starch-based bioplastic in anaerobic condition: Finding a proper kinetic model. *Bioresour. Technol.* **2022**, *347*, 126661. [CrossRef]
19. Makut, B.B.; Das, D.; Goswami, G. Production of microbial biomass feedstock via co-cultivation of microalgae-bacteria consortium coupled with effective wastewater treatment: A sustainable approach. *Algal Res.* **2019**, *37*, 228–239. [CrossRef]
20. Yang, J.; Gou, Y.; Fang, F.; Guo, J.; Lu, L.; Zhou, Y.; Ma, H. Potential of wastewater treatment using a concentrated and suspended algal-bacterial consortium in a photo membrane bioreactor. *Chem. Eng. J.* **2018**, *335*, 154–160. [CrossRef]
21. Cao, J.; Zhu, Q.; Zhang, T.; Wu, Y.; Zhang, Q.; Fu, B.; Fang, F.; Feng, Q.; Luo, J. Distribution patterns of microbial community and functional characteristics in full-scale wastewater treatment plants: Focusing on the influent types. *Chemosphere* **2021**, *281*, 130899. [CrossRef] [PubMed]
22. Fan, J.; Chen, Y.; Zhang, T.C.; Ji, B.; Cao, L. Performance of Chlorella sorokiniana-activated sludge consortium treating wastewater under light-limited heterotrophic condition. *Chem. Eng. J.* **2020**, *382*, 122799. [CrossRef]
23. Ludwig, W. ARB: A software environment for sequence data. *Nucleic Acids Res.* **2004**, *32*, 1363–1371. [CrossRef]
24. Pirmoradi, N.; Ghaneian, M.T.; Ehrampoush, M.H.; Salmani, M.H.; Hatami, B. The conversion of poultry slaughterhouse wastewater sludge into biodiesel: Process modeling and optimization. *Renew. Energy* **2021**, *178*, 1236–1249. [CrossRef]
25. Xiong, J.-Q.; Kurade, M.B.; Kim, J.R.; Roh, H.-S.; Jeon, B.-H. Ciprofloxacin toxicity and its co-metabolic removal by a freshwater microalga Chlamydomonas mexicana. *J. Hazard. Mater.* **2017**, *323*, 212–219. [CrossRef] [PubMed]
26. Damergi, E.; Schwitzguébel, J.-P.; Refardt, D.; Sharma, S.; Holliger, C.; Ludwig, C. Extraction of carotenoids from Chlorella vulgaris using green solvents and syngas production from residual biomass. *Algal Res.* **2017**, *25*, 488–495. [CrossRef]
27. Jabir, T.F.; Noor Abbood, H.A.; Salman, F.S.; Hafit, A.Y. Influence of pH, pesticide and radiation interactions on the chemical composition of Chlorella vulgaris algae. *IOP Conf. Ser. Earth Environ. Sci.* **2021**, *722*, 012046. [CrossRef]
28. Drouin, G.; Droz, B.; Leresche, F.; Payraudeau, S.; Masbou, J.; Imfeld, G. Direct and indirect photodegradation of atrazine and S -metolachlor in agriculturally impacted surface water and associated C and N isotope fractionation. *Environ. Sci. Process. Impacts* **2021**, *23*, 1791–1802. [CrossRef]
29. de Wilt, A.; Butkovskyi, A.; Tuantet, K.; Leal, L.H.; Fernandes, T.V.; Langenhoff, A.; Zeeman, G. Micropollutant removal in an algal treatment system fed with source separated wastewater streams. *J. Hazard. Mater.* **2016**, *304*, 84–92. [CrossRef]
30. Presentato, A.; Lampis, S.; Vantini, A.; Manea, F.; Daprà, F.; Zuccoli, S.; Vallini, G. On the Ability of Perfluorohexane Sulfonate (PFHxS) Bioaccumulation by Two Pseudomonas sp. Strains Isolated from PFAS-Contaminated Environmental Matrices. *Microorganisms* **2020**, *8*, 92. [CrossRef]
31. García-Galán, M.J.; Matamoros, V.; Uggetti, E.; Díez-Montero, R.; García, J. Removal and environmental risk assessment of contaminants of emerging concern from irrigation waters in a semi-closed microalgae photobioreactor. *Environ. Res.* **2021**, *194*, 110278. [CrossRef]
32. Prosenc, F.; Piechocka, J.; Škufca, D.; Heath, E.; Griessler Bulc, T.; Istenič, D.; Buttiglieri, G. Microalgae-based removal of contaminants of emerging concern: Mechanisms in Chlorella vulgaris and mixed algal-bacterial cultures. *J. Hazard. Mater.* **2021**, *418*, 126284. [CrossRef] [PubMed]
33. Meng, L.; Wang, J.; Li, X.; Yu, Y.; Zhu, Y. Microbial community and molecular ecological network in the EGSB reactor treating antibiotic wastewater: Response to environmental factors. *Ecotoxicol. Environ. Saf.* **2021**, *208*, 111669. [CrossRef] [PubMed]
34. Matamoros, V.; Uggetti, E.; García, J.; Bayona, J.M. Assessment of the mechanisms involved in the removal of emerging contaminants by microalgae from wastewater: A laboratory scale study. *J. Hazard. Mater.* **2016**, *301*, 197–205. [CrossRef] [PubMed]
35. da Silva Ribeiro, T.; Grossi, C.D.; Merma, A.G.; dos Santos, B.F.; Torem, M.L. Removal of boron from mining wastewaters by electrocoagulation method: Modelling experimental data using artificial neural networks. *Miner. Eng.* **2019**, *131*, 8–13. [CrossRef]
36. Sharma, R.; Mishra, A.; Pant, D.; Malaviya, P. Recent adva nces in microalgae-based remediation of industrial and non-industrial wastewaters with simultaneous recovery of value-added products. *Bioresour. Technol.* **2021**, *344*, 126129. [CrossRef]
37. Akizuki, S.; Cuevas-Rodríguez, G.; Toda, T. Microalgal-nitrifying bacterial consortium for energy-saving ammonia removal from anaerobic digestate of slaughterhouse wastewater. *J. Water Process Eng.* **2019**, *31*, 100753. [CrossRef]
38. Wang, J.; Song, A.; Huang, Y.; Liao, Q.; Xia, A.; Zhu, X.; Zhu, X. Domesticating Chlorella vulgaris with gradually increased the concentration of digested piggery wastewater to bio-remove ammonia nitrogen. *Algal Res.* **2021**, *60*, 102526. [CrossRef]
39. Guo, Y.; Wang, J.; Shinde, S.; Wang, X.; Li, Y.; Dai, Y.; Ren, J.; Zhang, P.; Liu, X. Simultaneous wastewater treatment and energy harvesting in microbial fuel cells: An update on the biocatalysts. *RSC Adv.* **2020**, *10*, 25874–25887. [CrossRef]

40. Nguyen, T.-T.-D.; Bui, X.-T.; Nguyen, T.-T.; Hao Ngo, H.; Yi Andrew Lin, K.; Lin, C.; Le, L.-T.; Dang, B.-T.; Bui, M.-H.; Varjani, S. Co-culture of microalgae-activated sludge in sequencing batch photobioreactor systems: Effects of natural and artificial lighting on wastewater treatment. *Bioresour. Technol.* **2022**, *343*, 126091. [CrossRef]
41. Zhu, S.; Qin, L.; Feng, P.; Shang, C.; Wang, Z.; Yuan, Z. Treatment of low C/N ratio wastewater and biomass production using co-culture of Chlorella vulgaris and activated sludge in a batch photobioreactor. *Bioresour. Technol.* **2019**, *274*, 313–320. [CrossRef] [PubMed]
42. Zhang, L.; Shen, Z.; Fang, W.; Gao, G. Composition of bacterial communities in municipal wastewater treatment plant. *Sci. Total Environ.* **2019**, *689*, 1181–1191. [CrossRef] [PubMed]
43. Nguyen, T.-T.-D.; Nguyen, T.-T.; An Binh, Q.; Bui, X.-T.; Ngo, H.H.; Vo, H.N.P.; Andrew Lin, K.-Y.; Vo, T.-D.-H.; Guo, W.; Lin, C.; et al. Co-culture of microalgae-activated sludge for wastewater treatment and biomass production: Exploring their role under different inoculation ratios. *Bioresour. Technol.* **2020**, *314*, 123754. [CrossRef] [PubMed]
44. Elmaadawy, K.; Liu, B.; Hassan, G.; Wang, X.; Wang, Q.; Hu, J.; Hou, H.; Yang, J.; Wu, X. Microalgae-assisted fixed-film activated sludge MFC for landfill leachate treatment and energy recovery. *Process Saf. Environ. Prot.* **2022**, *160*, 221–231. [CrossRef]
45. Maresca, J.A.; Keffer, J.L.; Hempel, P.P.; Polson, S.W.; Shevchenko, O.; Bhavsar, J.; Powell, D.; Miller, K.J.; Singh, A.; Hahn, M.W. Light Modulates the Physiology of Nonphototrophic Actinobacteria. *J. Bacteriol.* **2019**, *201*, e00740-18. [CrossRef]
46. Berthold, D.E.; Shetty, K.G.; Jayachandran, K.; Laughinghouse, H.D.; Gantar, M. Enhancing algal biomass and lipid production through bacterial co-culture. *Biomass Bioenergy* **2019**, *122*, 280–289. [CrossRef]
47. Ray, A.; Nayak, M.; Ghosh, A. A review on co-culturing of microalgae: A greener strategy towards sustainable biofuels production. *Sci. Total Environ.* **2022**, *802*, 149765. [CrossRef]
48. Anyanwu, R.C.; Rodriguez, C.; Durrant, A.; Olabi, A.G. Evaluation of Growth Rate and Biomass Productivity of Scenedesmus quadricauda and Chlorella vulgaris under Different LED Wavelengths and Photoperiods. *Sustainability* **2022**, *14*, 6108. [CrossRef]
49. Li, Y.; Liu, X.; Zheng, X.; Yang, M.; Gao, X.; Huang, J.; Zhang, L.; Fan, Z. Toxic effects and mechanisms of PFOA and its substitute GenX on the photosynthesis of *Chlorella pyrenoidosa*. *Sci. Total Environ.* **2021**, *765*, 144431. [CrossRef]