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Enhancement of carbamazepine removal rate using *Tetradesmus obliquus* KNUA061 and NaOCl and utilization of the resulting biomass

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Pharmaceutical and personal care products (PPCPs) are discharged into receiving water bodies mainly from sewage treatment plants. Due to the inefficient removal in conventional wastewater treatment facilities, PPCPs have become a major concern to aquatic ecosystems, water quality, and public health worldwide since they cause harmful effects on aquatic life and human even at low doses. Among the PPCPs, carbamazepine (CBZ) is one of the most commonly prescribed anticonvulsant drugs and consumed more than 1,000 tons per year. Due to its structural complexity, CBZ is known as recalcitrant compound highly stable during wastewater treatment. Consequently, it has become one of the most frequently detected pharmaceuticals in waste water, surface water, and even drinking water. In this study, Korean indigenous microalgae strains were tested as eco-friendly and cost-effective solutions for CBZ removal. Based on the preliminary biological CBZ degradation tests, *Tetradesmus obliquus* KNUA061 demonstrating the best CBZ removal rate was selected for further experiments. In order to increase strain KNUA061's CBZ removal efficiency, NaOCl, which is widely accepted in the water purification process, was used as an additional stimulus to induce stress conditions. At around 20 μg L^{-1} CBZ, addition of 1.0 mg NaOCl resulted in approximately 20% of removal rate increase without suppressing cells growth. Roughly 90% of CBZ remained its original form and the composition of the transformed secondary metabolites was less than 10% during the biodegradation process by the microalga. Based on the results of the antioxidant enzyme activities, degree of lipid oxidation, and amino acid contents, it was concluded that the redox-defence system in microalgal cells may have been activated by the NaOCl treatment. Biomass analysis results showed that higher heating value (HHV) of strain KNUA061 biomass was higher than those of lignocellulosic energy crops suggesting that it could be utilized as a possible renewable energy source. Even though its biodiesel properties were slightly below the international standards due to the high PUFA contents, the biodiesel produced from *T. obliquus* KNUA061 could be used as a blending resource for transportation fuels. It was also determined that the microalgal

biomass has acceptable feasibility as a sustainable dietary supplement feedstock due to its high essential amino acid contents.

KEYWORDS

microalgae, emerging micropollutant, carbamazepine, sodium hypochlorite, water treatment, biomass

1 Introduction

Emerging micropollutants can be divided categories such as personal care products, pesticides, pharmaceuticals, plasticizers, and preservatives, which are produced and consumed thousands of tons per year and released into various ecosystems (Rempel et al., 2021). PPCPs are defined as "any product used by individuals for personal health or cosmetic reasons." by the United States Environmental Protection Agency. All PPCPs have complex molecules and they comprise a diverse group of chemicals including all human and veterinary drugs and non-medicinal consumer chemicals such as personal hygiene products and household cleaning detergents. PPCPs are released to the aquatic environments by multiple sources including human excretion in urine and feces, discharge from pharmaceutical manufacturers, and residues from agricultural activities (Al-Baldawi et al., 2021). Among all these sources, human contribution by discharge into the sewer systems is one of the major PPCPs sources in the environment. Thereby, the main pollutant source of PPCPs remaining in surface water that is used as drinking and irrigation water is sewage treatment effluent.

Due to the improvement of living standards, a large amount of medicines and personal hygiene substances have been discharged into the water system, resulting in an increase in the frequency of detection and concentration of PPCPs every year (Boxall et al., 2012). Although not every PPCP is necessarily detrimental to human and aquatic organisms, numerous studies have demonstrated that some of these chemicals could act as toxicants to a variety of biological processes in ecosystems (Chee-Sanford et al., 2001; Ash et al., 2002; Costanzo et al., 2005; Chen et al., 2008; Hoppe et al., 2012; Rosi-Marshall and Royer 2012; Rosi-Marshall et al., 2013; Richmond et al., 2017).

Because of the recalcitrant physicochemical properties of PPCPs, a complete removal of PPCPs by conventional wastewater treatment processes is not feasible and thereby their presence in drinking water poses a potential threat to aquatic life and public health (Snyder 2008; Wee et al., 2020). It has been reported that PPCPs and their secondary metabolites are biologically active and they can be accumulated in aquatic organisms especially in fish (Ebele et al., 2017; Muir et al., 2017; Yin et al., 2017). Hence, water quality management to reduce the concentration of PPCP in inflow sewage and discharged water and preventive measures for recalcitrant PPCP pollutants with high detection frequency and concentration are required.

Priority lists of pollutants have been developed by the United States Environmental Protection Agency (USEPA) and European Union (EU) aimed at identifying substances that may pose a risk to receiving waters and require regulation since 1970s. In 2007, PPCPs such as diclofenac, iopamidol, musks, and carbamazepine were identified as future emerging priority candidates (Ellis, 2008; Ebele et al., 2017). Then, the European Commission established regulatory guidelines (Directive 2013/39/EU) to monitor the pharmaceuticals in aquatic environments and ibuprofen, clofibric acid, triclosan, phthalates, and bisphenol A were also added to this watch list (EU Decision 2018/840, Loos et al., 2018; Alaoui et al., 2021).

PPCPs are either non-volatile or semi-volatile (Delorenzo and Fleming 2008) and they are generally hydrophilic compounds with a high water solubility (Ohoro et al., 2022). These physiochemical properties of PPCPs indicate that their distribution in nature mainly occurs through aqueous transport (Caliman and Gavrilescu 2009). PPCPs enter the environment as they pass-through the human body or when unused and/or unwanted PPCPs are improperly disposed of down the drain. Consequently, PPCPs are found virtually everywhere where domestic wastewater is discharged. Therefore, municipal wastewater treatment plants play a crucial role in safeguarding our ecosystems and public health. In the waste water treatment facilities, transformation of PPCPs occur depending on the structures and properties of each compound and the conditions of the operations. During the process, they may be completely or partially broken down or may remain intact in some cases (Xia et al., 2005; Kosma et al., 2014; Yang et al., 2017). In general, PPCP compounds that are frequently detected in wastewater effluents were recalcitrant or only partially degraded by conventional wastewater treatment facilities (Reyes et al., 2021). Previous studies have shown that PPCP removal during primary wastewater treatment which include coagulation, flocculation, and sedimentation is relatively insignificant (Oluwole et al., 2020). More advanced and complex wastewater treatment approaches which include membrane bioreactors, sand filtration, ultra-, micro-, and nanofiltration, reverse osmosis, activated carbon, and chemical oxidation *via* ozonation may enhance PPCP removal rates from wastewater, but these processes are chemically and operationally intensive and therefore they require large systems, infrastructure, and high operational costs (Oulton et al., 2010).

PPCPs can be degraded *via* abiotic transformation including hydrolysis, photodegradation, reductive catalysis,

volatilization, and ozonation (McDowell et al., 2005; Kim and Tanaka 2009; Calza et al., 2012; König et al., 2016; Maldonado-Torres et al., 2018; Wang et al., 2020b; Bonnot et al., 2022) and biological transformation such as bioaccumulation, adsorption, and intracellular and extracellular biodegradation by a verity of microorganisms such as bacteria, fungi, and microalgae (Kang et al., 2008; Jelic et al., 2012; Hena et al., 2021; Morais et al., 2021; Narayanan et al., 2022). Recently, microalgae-based PPCP bioremediation has gained increasing scientific attention due to its low operational costs and carbon-neutral natures (Hassaan et al., 2017; Daneshvar et al., 2018). Microalgae offer several advantages over bacteria and fungi as they do not require carbon sources for growth and PPCP breakdown and various microalgal species are capable of bioremediation of PPCPs (Abdelfattah et al., 2023).

Among many PPCPs, carbamazepine (CBZ) is one of the most widely used antiepileptic drugs in the world to treat neurosis and bipolar disorder (Mojiri et al., 2021). Approximately 1,014 tons of CBZ are consumed annually worldwide (Zhang et al., 2008). Consequently, CBZ that cannot be completely metabolized by the human body usually flow into receiving water bodies and it is one of the most frequently detected PPCPs in wastewater treatment plants around the world (Zhang et al., 2008; Zhang et al., 2018; Seo et al., 2020; Huang et al., 2022; Ma et al., 2022). However, CBZ has been reported to have very low removal efficiency (7%–10%) in conventional wastewater treatment facilities (Zhang et al., 2008) due to its relatively low attenuation rate during both abiotic and biotic processes (Baena-Nogueras et al., 2017; Thanekar et al., 2018; Dao et al., 2022). Many researches have been attempted related to the chemical oxidation of CBZ in aqueous solution in conventional advanced oxidation processes *via* the generation of strong oxidizing oxygen species such as O_3/UV/H_2O_2 (Im et al., 2012; Esquerdo et al., 2021; Schoenell et al., 2022), UV/H_2O_2 (Vogna et al., 2004; Keen et al., 2012; Liu et al., 2015; Alharbi et al., 2017), photocatalytic (Doll and Frimmel 2005; Xu et al., 2013; Carabin et al., 2016; Yang et al., 2019), and photo-Fenton (Klamerth et al., 2010; Guo et al., 2021; Villota et al., 2021; Gong et al., 2022; Sun et al., 2022). Some of these methods achieved a 100% removal rate from different concentrations of CBZ in wastewater (Supplementary Table S1). However, these technologies use harsh chemicals and some of the catalysts may act as additional contaminants in the aquatic environments (Mohapatra et al., 2014). Moreover, higher capital and operating costs and energy input and generation of additional toxic by-products attribute for the limited acceptance of the advanced oxidation processes in CBZ treatment (Wang and Zhu 2016; Zhai et al., 2018; Wang et al., 2020a). Hence, it is needed to find improved remediation solutions for CBZ-contaminated wastewater that may threat aquatic ecosystems and human's health.

In this study, CBZ removal screening was conducted using 12 microalgal strains previously isolated by our research group from primary settled wastewater in the local municipal wastewater treatment plants. The removal rate of CBZ and its secondary metabolites were analyzed using the selected elite microalgal strains. In addition, it was investigated whether bioremediation efficiency could be increased by stimulating the microalgal redox system. NaOCl was used as a stressor as it is generally used for sterilization in water treatment. The physiological and biochemical characteristics of the resulting microalgal biomass were investigated to examine its potential for use as bioenergy and high-value products.

2 Materials and methods

2.1 Chemicals

All the chemicals used in this study were analytical grade. CBZ (purity: ≥ 99.9%) was purchased from Sigma-Aldrich (St. Louis, MO, United States) and NaOCl was obtained from Duksan (Ansan, Korea), respectively. Methanol from Merck (Darmstadt, Germany) and carbamazepine-10,11-epoxide (EP-CBZ), 10,11-dihydrocarbamazepine (DI-CBZ), 9-acridine carboxaldehyde (= acridine-9-carbaldehyde), 3-hydroxy carbamazepine (3-OH-CBZ), and 10,11-dihydro-10-hydroxy carbamazepine (DiOHCBZ) manufactured by Sigma-Aldrich were used in this research.

2.2 Selection of microalgal strains and growth conditions

In the preliminary experiments, a total of 13 microalgal strains were tested for their CBZ-degrading capabilities at a concentration of approximately 1.0 mg L^{-1} CBZ (Supplementary Table S6). Four best microalgae with the highest removal rates were selected and test for the second CBZ degradation tests at a concentration of about 250 μg L^{-1} (Supplementary Table S7). *Tetradesmus obliquus* strain KNUA061 isolated from a wastewater treatment plant was finally chosen as the best candidate for further CBZ removal experiments. Each culture was inoculated in 1 L Erlenmeyer flasks containing 500 ml BG-11 medium (Sigma-Aldrich) with an initial CBZ concentrations of around 50 μg L^{-1} and then 20 μg L^{-1}. All samples were cultivated at 25°C with shaking at 120 rpm and supplying with 1% CO_2 under approximately 100 μmol m^{-2} s^{-1} of light intensity in 16-h/8-h light/dark cycle. NaOCl was added as a stimulant to enhance CBZ removal efficiency at three different concentrations (1 mg L^{-1}, 5 mg L^{-1}, and 10 mg L^{-1}) that

did not significantly inhibit microalgal growth as confirmed in the preliminary tests.

2.3 Microalgal growth characteristics

Microalgal biomass productivity was measured as dry weight (DW) using a gravimetric method proposed by the American Public Health Association. Microalgal growth rates were monitored by measuring the optical density (OD at 680 nm) at 5 days intervals using an Optimizer 2120 UV spectrophotometer (Mecasys, Daejeon, Korea). In addition, pH was measured using a pH meter (Seven Easy, Mettler Toledo, Columbus, OH, United States) to determine the effects of CBZ and NaOCl on microalgal cultivation.

Microalgal growth rates were monitored by measuring the optical density (OD at 680 nm) and specific growth rate (μ) calculated as: $\mu = \ln (N_2/N_1)/(t_2-t_1)$, where N_2 and N_1 refer to optical density at times t_2 and t_1 during the exponential growth phase (Levasseur et al., 1993; Humphrey et al., 2021).

After 20 days of cultivation, chlorophyll *a*, chlorophyll *b*, and total carotenoid contents were measured. Two milliliters of the culture was centrifuged (Centrifuge 5424, Eppendorf, Hamburg, Germany) and washed twice with distilled water. The harvested cell pellets were suspended in 2 ml of methanol and disrupted in an ice bath using ultrasonication (550 Sonic Dismembrator, Fisher Scientific, Hampton, NH, United States) for 5 min. The extracted solution was incubated at 4°C for 24 h in the dark and centrifuged at 16,022 *g* for 10 min. The cleared supernatant was measured using a spectrophotometer at 470, 653, and 666 nm. Pigment contents were calculated using the following equations (Wellburn, 1994):

$$\text{Chlorophyll } a\ (C_a) = 15.65\ A_{666} - 7.34\ A_{653},$$
$$\text{Chlorophyll } b\ (C_b) = 27.05\ A_{653} - 11.21\ A_{666}, \text{ and}$$
$$\text{Total carotenoid} = (1,000\ A_{470} - 2.86\ C_a - 129.2\ C_b)/221.$$

2.4 Measurement of CBZ removal efficiency and metabolites

Abiotic controls were included in the experiments to investigate photolysis-driven and NaOCl-driven degradations of CBZ along with the microalgae cultures supplemented with different levels of NaOCl as a stimulator (Figure 2). The samples were collected every 5 days, filtered through a 0.20 μm membrane filter (Sartorius Minisart, Göttingen, Germany), and subjected to high-performance liquid chromatography (HPLC; Agilent 1,200 series, Agilent, Santa Clara, CA, United States) with a mobile phase of 40:60 (v/v) water and methanol. The solution was separated using a SunFire column C18 (2.4 × 250 mm, 5 μm, (Waters, Milford, MA, United States) at a flow rate of 1 ml min^{-1}, column temperature of 40 ± 1.0°C, and sample injection volume of 5 μL. CBZ was detected using a diode array detector set to 285 nm. All experiments were conducted in triplicate.

For secondary metabolite analysis, strain KNUA061 was cultivated in BG-11 medium supplied at 20 μg L^{-1} CBZ without NaOCl (control) and with 1 mg L^{-1} NaOCl (treatment group), respectively. Acquity ultra high sensitivity (UPLC) H-Class Plus system with Xevo TQ-S micro triple quadrupole mass spectrometry (Waters) was used to analyze CBZ and its metabolites with a mobile phase of 50:50 (v/v) water and acetonitrile (Sigma-Aldrich) with 0.1% formic acid (Sigma-Aldrich). Chromatographic separation of the target compounds was achieved on an Acquity BEH C18 column (2.1 × 100 mm, 1.7 μm, Waters) at a flow rate of 0.2 ml min^{-1} and column temperature at 40°C ± 5.0°C.

2.5 ROS-related redox system

2.5.1 Measurement of ROS production

Oxidation-sensitive fluorescent probe, H2DCFDA, was used to analyze intracellular ROS levels according to previously reported publication by Astuya et al. (2018), except for washing the cells with phosphate-buffered saline (PBS). PBS is a widely used solution in many biological research applications before fluorescence dye staining since it is isotonic and non-toxic to cells (Tripathi et al., 2015; Jeyakumar et al., 2020; Yan et al., 2021; Nigam et al., 2022). Cells treated with NaOCl and CBZ were harvested, washed twice with PBS (Sigma-Aldrich), and incubated at a final concentration of 5 μMH2DCFDA at 30°C for 30 min in the dark. The cells were then centrifuged, washed twice with PBS, and diluted with 1 ml PBS. Fluorescence images were obtained using a super-resolution confocal laser scanning microscope (LSM 800 with Airy scan, Carl Zeiss) for H2DCFDA (green; excitation at 495 nm, emission at 520 nm). The fluorescence was also analyzed using flow cytometric analysis with a BD FACSAria III (Becton Dickinson and Company, New Jersey, NJ, United States) using 10,000 events.

2.5.2 Measurement of redox status

Microalgal samples were harvested after 24 h with 1 mg L^{-1} NaOCl and approximately 20 μg L^{-1} CBZ and centrifuged at 1,516 *g* for 10 min at 4°C. The pellet was resuspended in PBS, extracted in an ice bath using a probe-type sonicator (Sonics Vibra Cell VC 505, Sonics & Materials, Newtown, CT, United States), and centrifuged at 16,022 *g* for 10 min at 4°C. The supernatant was transferred to a new tube and used for the antioxidant enzyme assays. A superoxide dismutase (SOD), catalase (CAT), and peroxidase (PER) activities in the samples were measured using BO-SOD-250, BO-CAT-400, and BO-PER-500 assay kits (Biomax, Seoul, Korea), respectively, according to the manufacturer's instructions.

To confirm the non-enzymatic antioxidant system, the TBARS assay was conducted using a TBARS assay kit (BO-TBR-200, Biomax) in order to observe the change in MDA content as an indicator of lipid peroxidation. BODIPY 581/591 was used to analyze intracellular lipid peroxidation levels, as described previously (Domínguez-Rebolledo et al., 2010). Cells were treated with 1 mg L^{-1} NaOCl and 20 μg L^{-1} CBZ, harvested, washed twice with PBS, and incubated at a final concentration of 5 μM BODIPY 581/591 at 25°C for 30 min in the dark. The cells were then centrifuged, washed twice with PBS, and diluted with 1 ml PBS. Flow cytometric analysis was conducted using BD FACSAria III with 10,000 events (excitation at 488 nm, emission at 530 nm).

2.6 Characteristics of the resulting microalgal biomass

2.6.1 Proximate and ultimate analyses

Microalgal cultures were harvested after 20 days of cultivation. Biomass samples of the non-treated control, CBZ treatment, and CBZ + NaOCl treatment groups were prepared to compare their characteristics. The harvested microalgal biomass was lyophilized, pulverized, and sieved through an ASTM 230 mesh (63 μm, Chunggye, Seoul, Korea). Pulverized biomass was used for further analysis. During proximate analysis, 10 mg of biomass was used to measure moisture, volatile matter, and ash content using a thermal analyzer (Shimadzu, Kyoto, Japan). Nitrogen gas was supplied at a rate of 25 ml min^{-1}, and the biomass was heated from 50°C to 900°C at a rate of 10°C min^{-1}.

Ultimate analysis was conducted with a Flash 2000 elemental analyzer (Thermo Fisher Scientific, Milan, Italy) to determine C, H, N, and S contents. The oxygen (O) content was calculated by subtracting the contents of ash and CHNS from the total contents.

The HHV was determined using an equation developed by Given et al. (1986), where C, H, and S contents are provided by the elemental analysis and O content from the proximate analysis.

$HHV\ (MJ\ kg^{-1}) = 0.3278C + 1.419H + 0.09257S - 0.1379O + 131N + 0.637.$

2.6.2 Biochemical composition of the microalgal biomass

To determine carbohydrate content, 50 mg of freeze-dried biomass was hydrolyzed with 2.5 ml 2 N sulfuric acid (Sigma-Aldrich) at 94°C for 3 h (Jo et al., 2020b). Then the reaction tubes were cooled to ambient temperature, 40% $CaCO_3$ (Sigma-Aldrich) was added to the hydrolysates to neutralize the reaction. Samples were then filtered through a 0.2-μm polytetrafluoroethylene (PTFE) filter (Whatman, Florham Park, NJ, United States) and soluble carbohydrate contents (sucrose, maltose, lactose, glucose, xylose, galactose, fructose, fucose, and arabinose) were determined by HPLC with refractive index detector (HPLC-RI, Shimadzu, Kyoto, Japan). The total carbohydrate content was determined using the phenol-sulfuric acid method described by Mecozzi (2005).

The nitrogen (N) content was determined using ultimate analysis, and the total protein content was calculated from the N content using the conversion factor (× 6.25) (Mariotti et al., 2008). Biomass powder (500 mg) was used to quantitatively determine free amino acid content using an amino acid analyzer (L-8900, Hitachi, Tokyo, Japan).

Total lipid content was measured using the sulfo-phospho-vanillin method (Mishra et al., 2014) and fatty acid methyl ester (FAME) was extracted according to the method described by Breuer et al. (2013) with slight modifications. The harvested biomass samples were freeze-dried and pulverized to enhance the extraction efficiency. Ten mg of biomass was placed in a 1.5 ml tube and the same volume of glass beads (1 mm, Glass Beads 1; Glastechnique, Wertheim, Germany) was added to the tube. Then, 1 ml of $CHCl_3$:MeOH mixture (4:5, v/v, Sigma-Aldrich) was added to the tube and it was bead-beated 8 times in a microtube mixer (MicroMixer E-36, Taitec, Saitama, Japan) for 1 min. The solution was transferred to a 11 ml glass tube and it was vortexed for 5 s and sonicated at 40 kHz and 60°C in an ultrasonic bath (Branson 5800, Branson Ultrasonics, Sterling Heights, MI, United States) for 10 min. Then, 2.5 ml of distilled water containing 50 mM Tris (Sigma-Aldrich) and 1 M NaCl (Duksan) was added to the glass tube and it was vortexed and sonicated again. After the second sonication, the glass tubes were centrifuged for 5 min at 1,200 *g* and the chloroform phase at the bottom was transferred to a new glass tube using a glass Pasteur pipette (Hilgenberg, Malsfeld, Germany). To further extract the remaining lipid, 1 ml of chloroform was added to the previous tube. Vortexing, sonication, and centrifugation steps were repeated. Collected chloroform phases were evaporated in a rotary evaporator (RV 10, IKA, Staufen, Germany). For transesterification to FAME, 3 ml of methanol containing 5% (v/v) sulfuric acid (Sigma-Aldrich) was added to the glass tube containing the dried extracted lipids and vortexed for 5 s. The tube was then incubated for 3 h at 70°C in a water bath (Daihan Labtech, Namyangju, Korea) with vortexing the tube every 30 min. The tube was cooled down to room temperature and 3 ml of distilled water and 3 ml of hexane were added to glass tubes. Then, it was vortex for 5 s and mixed on an orbital shaker (TW3, FinePCR, Gunpo, Korea) for 15 min. After shaking, the glass tubes were centrifuged for 5 min at 1,200 *g* and 2 ml of the hexane phase on the top layer was transferred into a new glass tube. In order to wash the collected hexane phase, 2 ml of distilled water was added, vortexed for 5 s, and centrifuge for 5 min at 1,200 *g*. The hexane phase was then filtered through a 0.2 μm polyvinylidene fluoride (PVDF) syringe filter (Chromdisc, Hwaseong, Korea). FAME composition was analyzed using gas chromatography (GC) mass spectrometry (Agilent 7890A GC equipped with 5975C MSD; Agilent) with a DB-FFAP column (30 m × 250 μm × 0.25 μm, Agilent).

Biodiesel quality was determined by assessing the degree of unsaturation (DU), long-chain saturated factor (LCSF), saponification value (SV), iodine value (IV), cetane number

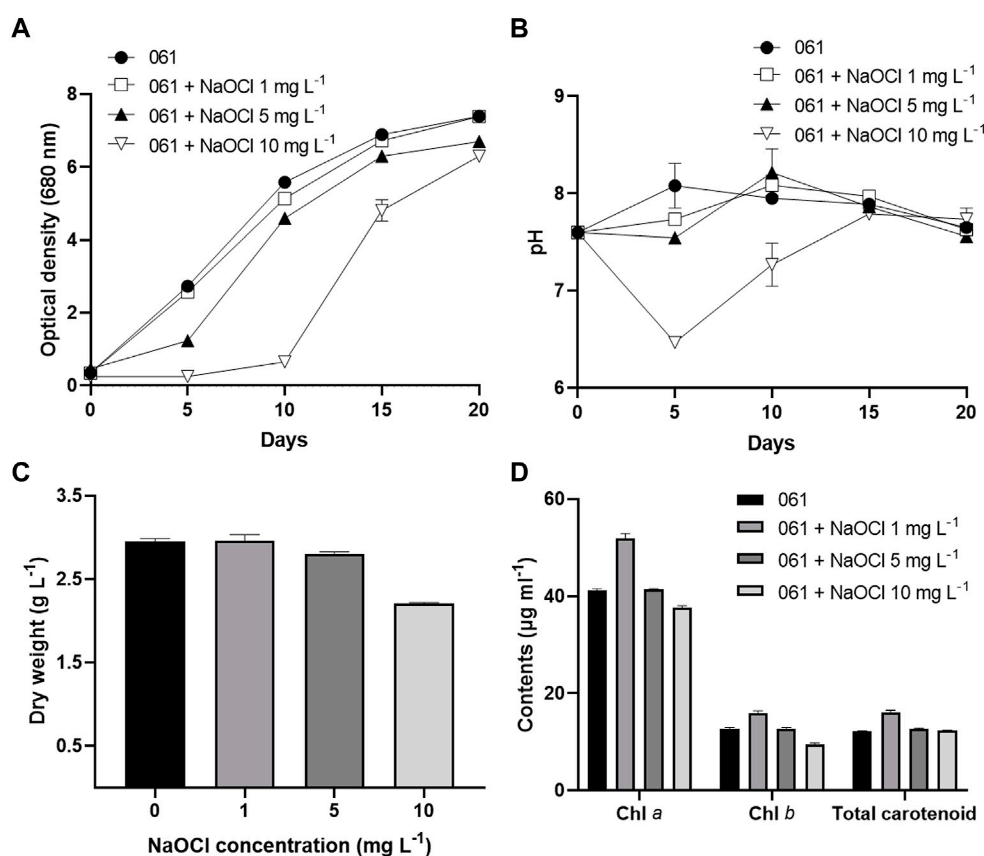

FIGURE 1
Growth characteristics of *T. obliquus* KNUA061 under different NaOCl concentrations. OD **(A)** and pH **(B)** of *T. obliquus* KNUA061 during the 20 days of cultivation. DW **(C)** and chlorophyll and total carotenoid contents **(D)** of microalgal biomass after 20 days of cultivation.

(CN), cold filter plug-going point (CFPP), kinematic viscosity at 40°C (υ), density at 20°C (ρ), and oxidative stability, which were all calculated based on the fatty acid profiles, using the equations below; EN 14214 were the standards used to evaluate biofuel quality (Sakthivel et al., 2018):

$$SV = \sum \frac{560 \times F}{MW},$$

$$IV = \sum \frac{254 \times F \times N}{MW},$$

$$CN = \left(46.3 + \frac{5458}{SV}\right) - (0.225 \times IV),$$

$$LCSF = (0.1 \times C16) + (0.5 \times C18) + (1 \times C20) + (1.5 \times C22) + (2 \times C24)$$

$$CFPP = (3.1417 \times LCSF) - (16.477)$$

$$DU = \sum MUFA + (2 \times PUFA)$$

$$\ln(\upsilon) = \sum -12.503 + 2.496 \times \ln(MW) - 0.178 \times N$$

$$\rho = \sum 0.8463 + \frac{4.9}{MW} + 0.0118 \times N, \text{ and}$$

$$\text{Oxidative stability} = \frac{117.9295}{X} + 2.5905\,(0 < 100).$$

Where MUFA is monounsaturated fatty acid, PUFA is polyunsaturated fatty acid, F is the percentage, MW is the molecular weight, N is the number of double bonds, and X is the content of linoleic and linolenic acids in each FAME value. These equations have been previously validated for use in the estimation of microalgae-based biofuel quality (Francisco et al., 2010).

2.7 Statistical analyses

All experiments were conducted in triplicate (at least). All data are indicated as the average of triplicate measurements, and error bars represent the standard deviation (SD). GraphPad Prism 9.0 (GraphPad Software, San Diego, CA, United States)

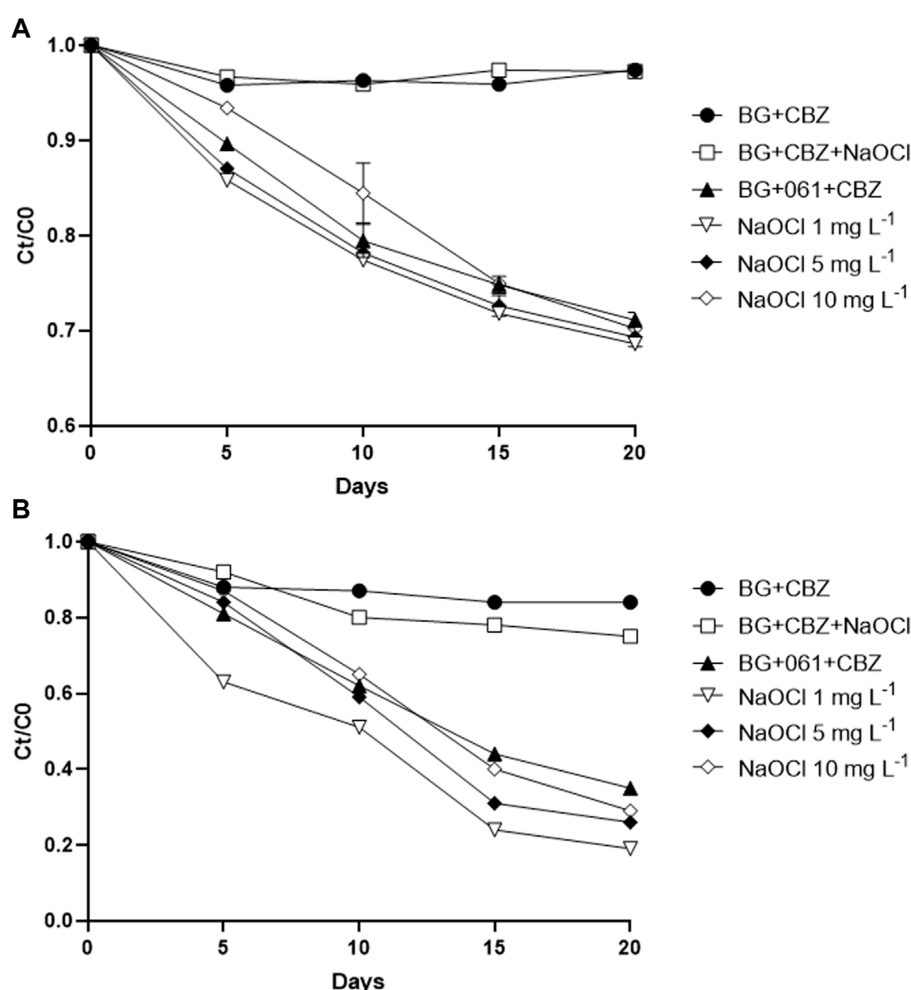

FIGURE 2
Removal of CBZ by *T. obliquus* KNUA061 with different concentrations of initial CBZ and NaOCl. **(A)** 50 μg L^{-1} CBZ. **(B)** 20 μg L^{-1} CBZ.

was used to analyze the experimental data. One-way analyses of variance, followed by Dunnett's multiple comparison tests, was used to perform statistical analyses of the data. The significance level was set at 95% (*p-value* < 0.05).

3 Results

3.1 Microalgal strain and growth characteristics

The first preliminary test was carried out to screen elite microalgae with high CBZ removal rates among the 13 strains (Supplementary Figure S1; Supplementary Table S6). The second removal rate test was conducted using four best candidates (strains KNUA061, KNUA064, KNUA068, and KNUA069) that showed the highest removal rates in the first screening test (Supplementary Tables S6, S7). Among these microalgae, strain KNUA061 was found to be the most promising one (Supplementary Figure S1) and it was subjected to further CBZ-degradation tests. Next, NaOCl was used as a stressor to promote metabolic process in the microalga of interest and growth rate was measured under different concentrations of NaOCl. In this study, 1 mg L^{-1} NaOCl resulted in the lowest growth inhibition and highest growth rate (Supplementary Figure S2).

To improve CBZ removal rate of *T. obliquus* KNUA061, three different NaOCl concentrations (1 mg L^{-1}, 5 mg L^{-1}, and 10 mg L^{-1}) were used and growth characteristics of the microalga were monitored. OD results demonstrated that 1 mg L^{-1} NaOCl treatment achieved relatively high growth rate, almost similar to that of the control (without NaOCl addition), while 5 mg L^{-1} NaOCl-treated group showed slightly diminished growth rate. Growth rate increased fractionally in the 10 mg L^{-1} NaOCl treatment group until the 10th day of culturing and after that point exponential growth was recorded (Figure 1A). pH values

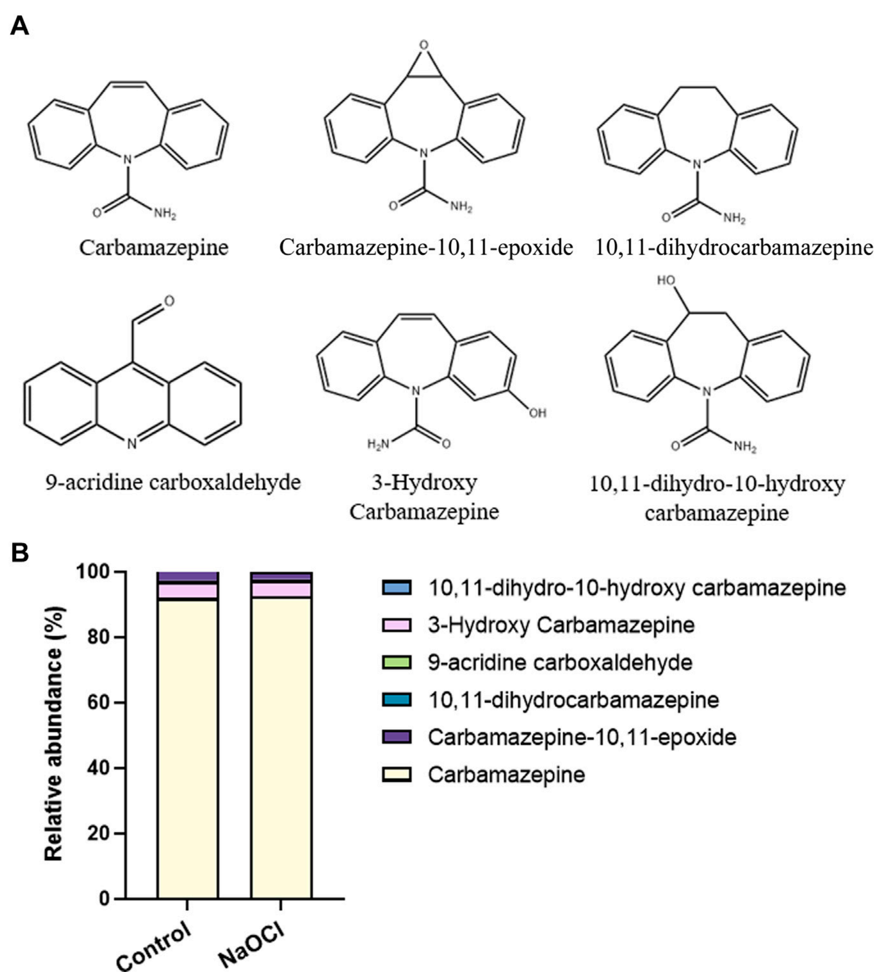

FIGURE 3
Relative abundance of CBZ and metabolites. **(A)** Chemical structures of CBZ and its 5 metabolites. **(B)** Relative abundance of CBZ and its metabolites with and without NaOCl treatment.

also showed similar patterns to those of the OD results. In particular, relatively low pH values were observed during the lag and exponential growth phases in the 10 mg L^{-1} NaOCl treatment (Figure 1B). DW was measured after 20 days of cultivation and the control and 1 mg L^{-1} NaOCl treatment groups demonstrated similar biomass productivities of 2.95 and 2.96 g L^{-1}, respectively. Productivities for 5 mg L^{-1} and 10 mg L^{-1} treatment groups were 2.81 and 2.21 g L^{-1}, respectively (Figure 1C). Chlorophyll and total carotenoid contents were the highest in 1 mg L^{-1} treatment group (Figure 1D).

3.2 CBZ removal efficiency and metabolites

CBZ removal rates were analyzed in *T. obliquus* KNUA061 cultures with different NaOCl concentrations. The highest removal rates were achieved by the microalgal culture with 1 mg L^{-1} NaOCl which also resulted in high growth rates when initial CBZ concentration of around 50 µg L^{-1} was used (Supplementary Table S4). The differences between the growth rates were narrowed in the later part of all the microalgal culture and around 30% of final CBZ removal rates were obtained in all the NaOCl treatment groups (Figure 2A and Supplementary Table S4). Since there is only a minute amount of CBZ exist in wastewater plants and surface waters in general, our second CBZ degradation experiment was conducted as low as at around 20 µg L^{-1}. More clear differences between the treatment groups were observed. Abiotic removal rates of the photolysis control and NaOCl groups were 14.6% and 19.5%, respectively, whereas the biotic control of strain KNUA061 only culture (without NaOCl) showed 62.5% of removal rate (Supplementary Table S5). Removal rates of the NaOCl-added microalgal culture groups were 82.0% for 1 mg L^{-1} NaOCl, 70.5% for 5 mg L^{-1} NaOCl, and 68.5% for 10 mg L^{-1} NaOCl, respectively (Figure 2B and Supplementary Table S5).

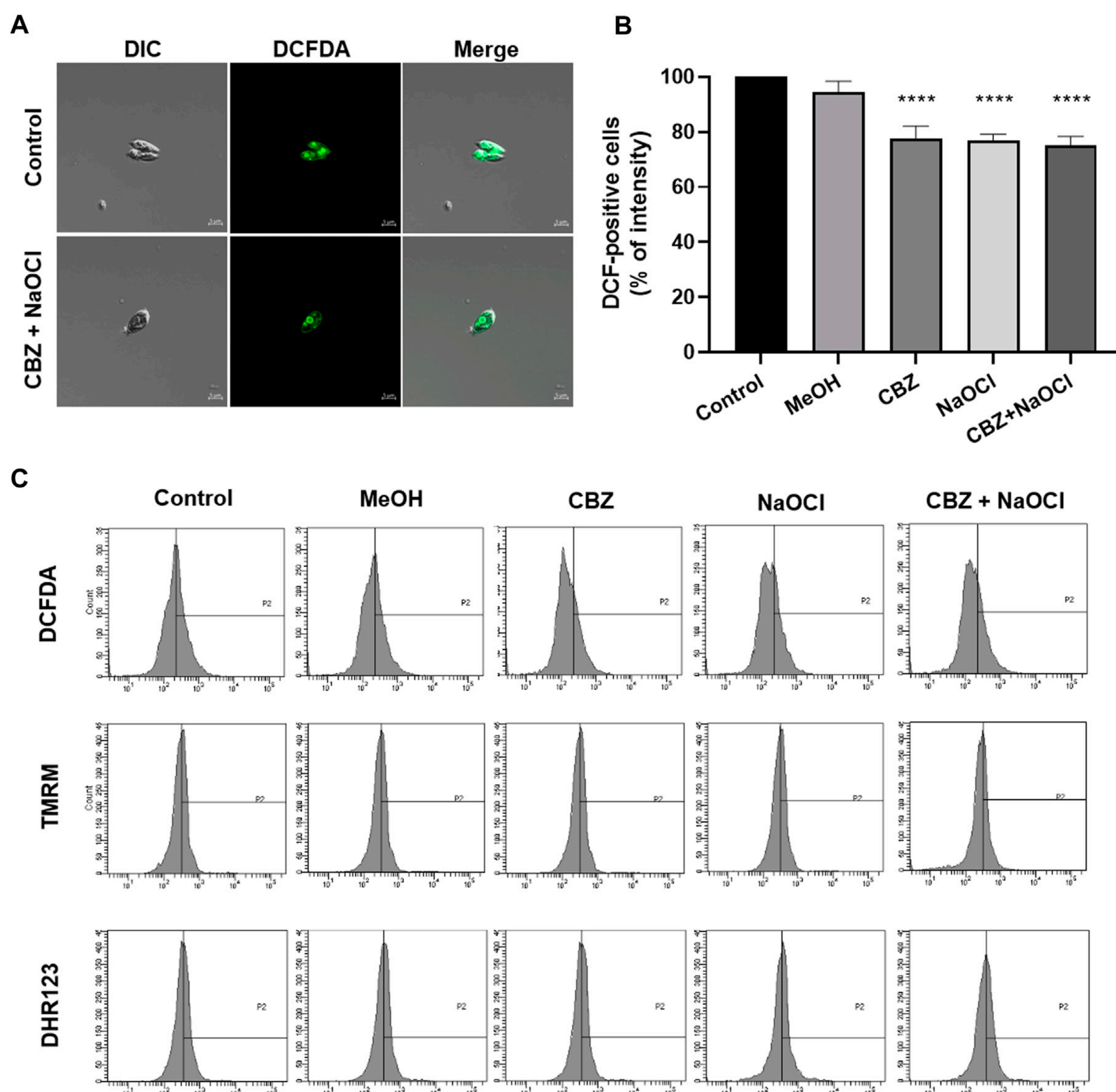

FIGURE 4
Determination of ROS content in *T. obliquus* KNUA061 after exposure to CBZ and NaOCl. **(A)** Confocal microscopy images of H2DCFDA-stained *T. obliquus* KNUA061; scale bars are shown in each image. **(B,C)** Relative intensity was measured using FACS after H2DCFDA, TMRM, and DHR123 staining. (**** p-value < 0.0001).

Secondary metabolites produced *via* the breakdown of CBZ in the microalgal cultures were also analyzed. The microalgal culture only control (without NaOCl) and 1 mg L^{-1} NaOCl treatment group were compared and CBZ, EP-CBZ, DI-CBZ, 9-acridine carboxaldehyde, 3-OH-CBZ, and DiOHCBZ levels were measured. While DiOHCBZ and 9-acridine carboxaldehyde were not found in either culture, approximately 4.9% of 3-OH-CBZ, 2.3%–2.6% of EP-CBZ, and 0.5% of DI-CBZ were detected in both groups (Figure 3).

3.3 ROS-related redox system during CBZ removal

Changes in total ROS content related to NaOCl addition was analyzed. ROS production levels were measured in the CBZ-, NaOCl-, and CBZ + NaOCl-treated groups by using H2DCFDA fluorescence intensity which indicated the total ROS content (Figure 4A). Fluorescence-activated cell sorting (FACS) analysis results showed a decrease in total ROS content after H2DCFDA staining, even though no

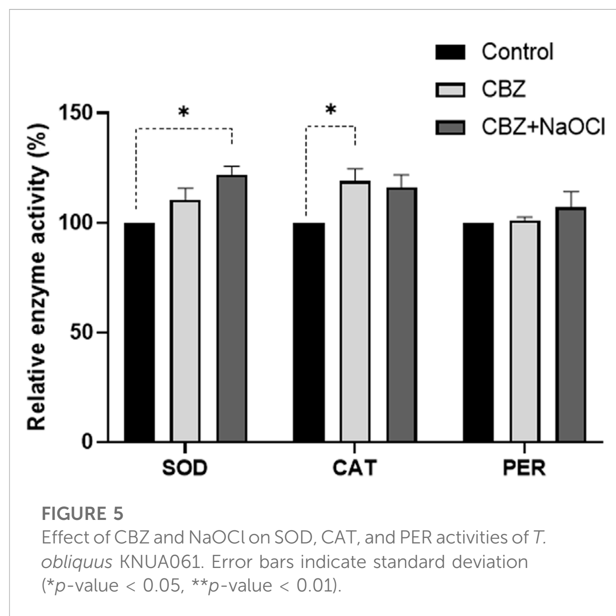

FIGURE 5
Effect of CBZ and NaOCl on SOD, CAT, and PER activities of *T. obliquus* KNUA061. Error bars indicate standard deviation (**p*-value < 0.05, ***p*-value < 0.01).

significant changes were detected by TMRM and DHR123 staining (Figures 4B,C).

Factors related to enzymatic and non-enzymatic redox systems were also investigated under CBZ and CBZ + NaOCl stress conditions. Compared to the non-treated control group, SOD, CAT, and PER activities, which are known as antioxidant enzymes primarily involved in ROS removal, increased by 10%, 19%, and 1% in the CBZ treatment and by 22%, 16%, and 7% in the CBZ + NaOCl treatment, respectively (Figure 5). The amount of MDA, which indicates the degree of lipid oxidation, decreased by 14% in the CBZ treatment group and by 16% in the CBZ + NaOCl group, respectively (Figure 6A). The fluorescence intensity, verified by BODIPY 581/591 staining, also decreased by 4.0% in the CBZ group and 10.8% in the CBZ + NaOCl treatment group, respectively (Figure 6B).

3.4 Characteristics of resulting microalgal biomass

All samples had more than 92.1% VM and less than 6.2% moisture content, respectively. The HHVs of all the samples calculated from the ultimate analysis results were similar to each other (approximately 23.3–23.1 MJ kg^{-1}) as listed in Table 1.

Biochemical analysis showed that total carbohydrate and lipid mass were the highest in the CBZ + NaOCl treatment group and the total protein content was the highest in the CBZ treatment group, respectively (Table 2). Monosaccharides compositions of all the treatment groups were also almost similar to each other (Supplementary Figure S3).

It was found that the CBZ treatment induced the highest total free amino acid content. Regarding essential amino acids, arginine showed the highest content of 0.666 mg g^{-1} in the control, while lysine was highest at 0.288 mg g^{-1} in the CBZ + NaOCl treatment. For non-essential amino acids, glutamic acid was in the highest (1.217 mg g^{-1}) in the CBZ + NaOCl treatment, whereas alanine was relatively low (0.477 mg g^{-1}) in the control. No cysteine was detected in any sample and proline was the lowest in the CBZ + NaOCl treatment (0.019 mg g^{-1}) as summarized in Table 3.

FAME analysis results demonstrated that palmitic acid (C16: 0), oleic acid (C18:1), linoleic acid (C18:2), and α-linolenic acid (C18:3 ω 3) were the major components of in all the 3 treatment groups. The PUFA contents were almost double to the saturated fatty acid (SFA) contents in the CBZ and CBZ + NaOCl treatment groups, while the highest total FAME content (93.52%) was observed in the CBZ + NaOCl-treated microalgal lipid. However, all three samples did not

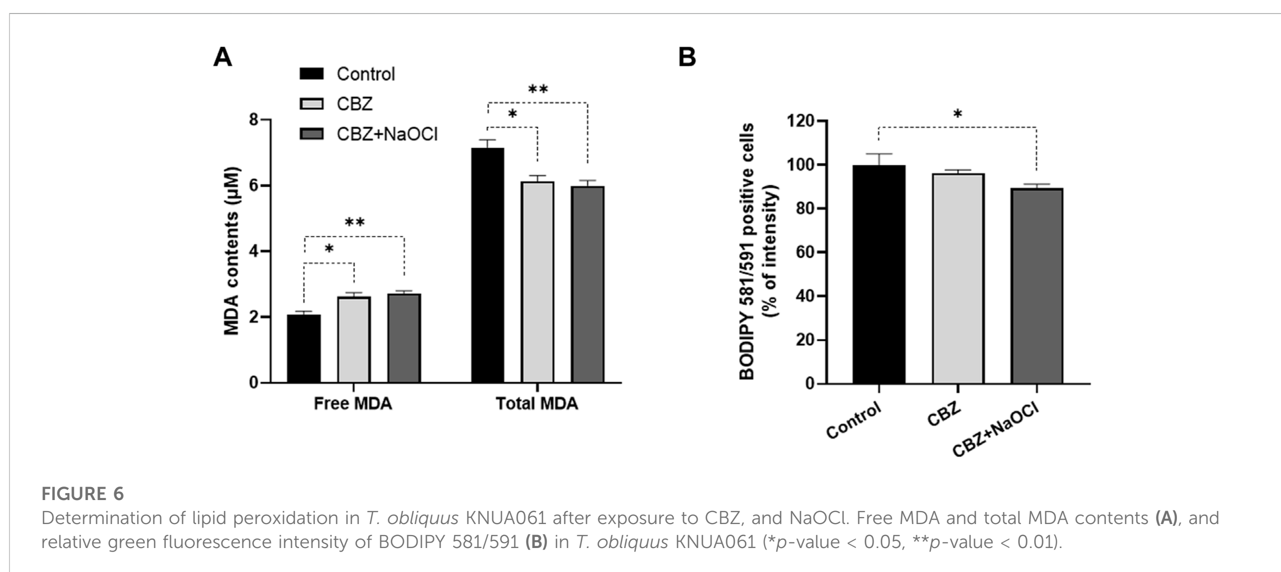

FIGURE 6
Determination of lipid peroxidation in *T. obliquus* KNUA061 after exposure to CBZ, and NaOCl. Free MDA and total MDA contents **(A)**, and relative green fluorescence intensity of BODIPY 581/591 **(B)** in *T. obliquus* KNUA061 (**p*-value < 0.05, ***p*-value < 0.01).

TABLE 1 Proximate and ultimate analyses of residual microalgal biomass.

	Control	CBZ	CBZ + NaOCl
Proximate analysis			
Moisture contents	5.68 ± 0.05	6.23 ± 0.22	5.86 ± 0.31
Volatile matter	93.04 ± 0.11	92.10 ± 0.24	92.63 ± 0.47
Fixed carbon + ash	1.29 ± 0.06	1.68 ± 0.40	1.52 ± 0.42
Ultimate analysis			
C	49.84 ± 0.01	49.41 ± 0	50.51 ± 0.09
H	7.57 ± 0.01	7.32 ± 0.02	7.56 ± 0.07
O	35.94 ± 0.05	35.79 ± 0.11	34.90 ± 0.15
N	5.08 ± 0.01	5.49 ± 0	5.21 ± 0
S	0.28 ± 0.01	0.32 ± 0.01	0.30 ± 0
HHV[a] (MJ kg^{-1})	22.8 ± 0	22.3 ± 0.1	23.1 ± 0.1

[a]Higher heating value.

TABLE 2 Biochemical composition of microalgal biomass.

	Control	CBZ	CBZ + NaOCl
Total carbohydrate	32.35 ± 4.06	34.16 ± 2.18	38.68 ± 1.60
Total protein	31.75 ± 0	34.31 ± 0	32.56 ± 0
Total lipid	19.14 ± 0.46	20.15 ± 1.23	22.90 ± 1.99

demonstrate statistically significant differences (Table 4). The biodiesel quality results were calculated based on the FAME results and there were no significant differences found between the samples. It was also confirmed that the IV and CN were not within the ranges of the EN14214 standard in all samples (Table 5).

4 Discussion

Our preliminary test of CBZ removal efficiency was conducted using 13 indigenous microalgal strains (Supplementary Table S2). *Desmodesmus intermedius* var. *balatonicus* KNUA024, which was isolated from the outdoor raceway pond at our mass cultivation facility located in Chilgok, Korea (Jo et al., 2020a), was chosen since this microalga exhibited high environmental stress resistance and excellent biomass productivity and wastewater treatment efficiency in our previous publications (Do et al., 2019; Do et al., 2021). The remaining 12 strains were isolated from the primary settled wastewater obtained from the local municipal wastewater treatment facilities in Daegu, Korea. Since various PPCPs may exist in wastewater treatment plants, they were tested as possible candidate strains as eco-friendly alternatives to wastewater treatment.

Before executing the preliminary experiments, the taxonomic positions for all the microalgal candidates were determined by both morphological and molecular characterizations. In particular, molecular identification results for strain KNUA061 by using three different marker genes such as internal transcribed spacer (ITS), 18S rRNA, and *rbc*L were conducted (Supplementary Table S3). In addition, the isolate's taxonomy as was cross-validated by morphological identification and classification. Since both morphological and molecular identification results indicated that strain KNUA061 belonged to the species *T. obliquus*, it was named as *T. obliquus* strain KNUA061. It should be noted that accurate species identification is essential for ecological research as well as safe and sustainable use of microalgae (Gantar and Svircev 2008; Champenois et al., 2015; Borowitzka 2016).

The first preliminary experiments were performed at a concentration of 1 mg L^{-1} CBZ with 13 strains and four most promising candidates were screened. Then, the second preliminary test was carried out with these top four strains at a concentration of 250 μg L^{-1} CBZ. Strain KNUA061 that demonstrated the best CBZ-degrading capability was finally selected for further study (Supplementary Figure S1).

Strain KNUA061 achieved a removal efficiency of approximately 11.4% in the second screening test at a concentration of around 250 μg L^{-1} CBZ (Supplementary Table S7). Then, NaOCl was used as a stressor to increase the removal efficiency. NaOCl is widely accepted for disinfection in the water treatment process because chlorination is a very simple, effective, and inexpensive way of wastewater purification (Kesar and Bhatti 2022). It also acts as a strong biocide that can eliminate pathogenic microorganisms and microalgae during water treatment (Emmanuel et al., 2004). Therefore, it was determined that up to which NaOCl concentrations *T. obliquus* KNUA061 cells still grow normally and its CBZ degrading metabolism can be activated under stress-inducing conditions. Growth rate of the microalga and its CBZ removal efficiency were investigated at 1 mg L^{-1}, 5 mg L^{-1}, and 10 mg L^{-1} NaOCl levels.

The microalga was cultured for 20 days and it was confirmed that the 1 mg L^{-1} NaOCl treatment group attained a high growth rate similar to that of the control (without NaOCl) and even produced the highest chlorophylls and carotenoids than others (Figure 1D). NaOCl treatment may have aided production of chlorophylls and carotenoids in strain KNUA061. In OD_{680}-and DW-based measurements, microalgal cells started to be affected at 1 mg L^{-1} NaOCl and cell growth was inhibited at 5 and 10 mg L^{-1} NaOCl indicating significant effect of chlorination on microalgal growth. At higher NaOCl concentrations, the cultures almost lost their chlorophylls at the early stage of cultivation, but a few cells that survived started to adapt the conditions and grew well at the later phases of cultivation as the effect of NaOCl in the culture media wore out (Figure 1A). This indicated that the concentrations of NaOCl used in this study

TABLE 3 Free amino acid contents of microalgal biomass with CBZ and NaOCl treatment.

	(mg g^{-1})	Control	CBZ	CBZ + NaOCl
Essential	Arginine	0.666	0.561	0.389
	Valine	0.040	0.053	0.061
	Isoleucine	0.000	0.022	0.029
	Leucine	0.023	0.022	0.023
	Phenylalanine	0.034	0.031	0.025
	Lysine	0.186	0.197	0.288
	Methionine	0.009	0.011	0.016
	Threonine	0.055	0.073	0.065
	Histidine	0.053	0.036	0.063
Non-essential	Aspartic acid	0.006	0.015	0.015
	Serine	0.094	0.117	0.073
	Tyrosine	0.037	0.018	0.024
	Glycine	0.039	0.038	0.030
	Alanine	0.477	0.696	0.613
	Cysteine*	0.000	0.000	0.000
	Glutamic acid*	0.742	1.201	1.217
	Proline*	0.033	0.037	0.019
	Hydroxy proline*	0.067	0.083	0.078
Total essential amino acids		1.067	1.006	0.063
Total non-essential amino acids		1.495	2.204	2.069
Total free amino acids		3.034	3.473	3.204

*Conditionally essential amino acid.

were not sufficient for completely eliminating the microalgal cells in the liquid culture media.

CBZ was gradually degraded during the microalgal cultivation and the removal rates of either photolysis or NaOCl alone in the abiotic controls were negligible (Figure 2; Supplementary Tables S4, S5). The removal rates were higher at 1 mg L^{-1} NaOCl than those of the microalga alone biotic controls (Supplementary Tables S4, S5). However, the difference of the removal rates gradually decreased in the later parts of the cultivation periods. In the groups treated with 10 mg L^{-1} NaOCl, the initial removal rates were insignificant, but improved rates were observed after 10 days of incubation. Nevertheless, the final removal rate was less than 30.0% in the first CBZ degradation test at 50.0 μg L^{-1} (Supplementary Table S4). As stated above, only minute concentrations of CBZ are present in surface water, the second CBZ removal experiment was conducted at approximately 20 μg L^{-1}. The second test results demonstrated that the removal rate in the 1 mg L^{-1} treatment was increased up to 82.0% (Supplementary Table S5). Considering the actual concentrations of CBZ detected in the rivers and wastewater treatment plants worldwide, higher removal efficiencies by using microalgae-based bioremediation can be expected. For instance, 1.1 μg L^{-1} CBZ and 13.8–145.0 ng L^{-1} CBZ were detected in the Han River and Nakdong River, respectively (Im et al., 2020; Seo et al., 2020).

To understand the CBZ removal process of microalgae, various methods have been used to determine the relative ratio of bioaccumulation, biosorption, and biodegradation (Hena et al., 2021). Five known secondary metabolites of CBZ including EPCBZ, DI-CBZ, 9-acridine carboxaldehyde, 3-OH-CBZ, and DiOHCBZ were prepared as standard intermediates and their compositions as breakdown products were compared in the control and 1 mg L^{-1} NaOCl-treated groups. Most of the remaining products after cultivation were in the original CBZ form and DiOHCBZ and 9-acridine carboxaldehyde were not detected at all. However, the compositions of 3-OH-CBZ (4.9%), DI-CBZ (0.5%), and EP-CBZ (2.3%–2.6%) were very similar to each other (Figure 3). Thus, addition of NaOCl to microalgal culture only increased the CBZ removal efficiency and there may be no significant changes occurred in the CBZ biodegradation pathways. However, it should be noted that EP-CBZ, which is one of the main metabolites of CBZ, was reported to be more toxic to *Chironomus riparius* (commonly known as the harlequin fly) than CBZ with an LC_{50} of 0.20 mg kg^{-1} (Heye et al., 2016), but its effects on the microalgal cells seemed neglectable due to very low concentrations. On the other hand, DI-CBZ was tested for its toxicity on zebrafish (*Danio rerio*) embryos, but no effect was observed (Pohl et al., 2020). To our knowledge, there is no information available on 9-acridine carboxaldehyde toxicity. It

TABLE 4 Fatty acid methyl ester composition of microalgal biomass.

	Control	CBZ	CBZ + NaOCl
C15:0	0.112	0.110	0.087
C16:0	23.558	22.611	22.702
C16:1	4.003	3.879	5.009
C16:2	2.412	1.946	3.186
C17:0	0.138	0.147	0.113
C16:3	3.690	3.899	4.716
C16:4	6.878	7.692	6.031
C18:0	1.090	0.830	0.874
C18:1	17.105	14.934	17.418
C18:2	12.083	13.829	14.116
C18:3 (ω6)	1.257	1.598	1.798
C18:3 (ω3)	15.878	16.858	14.066
C18:4	3.485	4.095	3.400
SFA[a]	24.76	23.55	23.66
MUFA[b]	21.11	18.81	22.43
PUFA[c]	45.68	49.92	47.31
Total FAME	91.69	92.43	93.52

[a]Saturated fatty acid.
[b]Monounsaturated fatty acid.
[c]Polyunsaturated fatty acid.

TABLE 5 Biodiesel properties of microalgal biomass calculated by fatty acid methyl ester composition.

	EN 14214	Control	CBZ	CBZ + NaOCl
SV	—	182.05	183.48	185.72
IV	≤120	137.96	147.43	140.24
CN	≥51	45.24	40.18	44.14
DU	—	112.5	118.6	117.1
LCSF	—	2.9	2.7	2.7
CFPP	≤ 5/≤ −20	−7.4	−8.1	−8.0
OS	≥6	6.6	6.2	6.5
υ	3.5–5.0	3.73	3.67	3.73
ρ	0.86–0.90	0.88	0.88	0.88

SV, saponification value; IV, iodine value (g I2 100 g-1 fat); CN, cetane number; DU, degree of unsaturation; LCSF, long-chain saturated factor; CFPP, cold filter plugging point (°C); OS, oxidation stability (h); υ, kinematic viscosity ($mm2s^{-1}$); ρ, Density ($g\ cm^{-3}$).

was demonstrated a higher acute toxicity of 3-OH-CBZ for *Vibrio fischeri* (Gram-negative marine bacterium) compared to the toxicity of CBZ (Kaiser et al., 2013; Bahlmann et al., 2014). In *V. fischeri* toxicity assay, DiOHCBZ exhibited almost unaltered toxicity compared to the its parental substrate (Kaiser et al., 2014). Overall, only minute amounts of harmful intermediates from CBZ were formed during the biodegradation process, but their toxic impact on the microalga is inconsequential.

NaOCl is a disinfectant which is widely used in water treatment and it was intended to activate the redox system by applying an appropriate level of NaOCl to the microalgal culture. Therefore, factors related to intracellular ROS regulation were investigated by treatment with 1 mg L^{-1} NaOCl, at which demonstrated a relatively high growth and the highest CBZ removal rate. Total ROS levels were reduced after CBZ, NaOCl and CBZ + NaOCl treatments (Figure 4). It was expected that the amount of total ROS would be reduced by the activation of the redox system in the microalga. DCFDA staining showed the decrease in ROS (Figures 4B,C) while, no significant difference was observed between the results from TMRM and DHR123 staining (Figure 4C). Antioxidant enzyme activities were measured to determine the factors that reduce ROS activities of SOD, CAT, and PER. These representative antioxidant enzymes were increased after the treatment with CBZ and CBZ + NaOCl (Figure 5). In particular, SOD activity was the highest in the CBZ + NaOCl treatment. These results indicated that CBZ and CBZ + NaOCl treatments somehow triggered the redox-defence system. Decreased MDA content and BODIPY 581/591 fluorescence intensity also showed that the CBZ and CBZ + NaOCl treatments decreased lipid peroxidation and also had some effect on ROS reduction (Figure 6). In conclusion, CBZ and 1 mg L^{-1} NaOCl NaOCl addition may have induced stress conditions that activate the ROS reducing mechanisms in the microalga without suppressing cell growth.

Amino acids are involved in a significant position of major metabolic processes. Non-essential amino acids are the naturally occurring ones and they do not need to be provided by an outside source since the human body can create them on its own. However, essential amino acids cannot be produced by the body, so they must be supplied from dietary proteins. Cysteine, glutamine, proline, and hydroxyproline are currently considered as conditionally essential amino acids that are non-essential amino acids that become essential under certain circumstances such as illness or pregnancy. Broadly speaking, microalgae have relatively high arginine and leucine contents and low methionine and histidine contents (Kolmakova and Kolmakov, 2019). Therefore, it can be inferred that an increase in lysine will result in advantageous fatty acid metabolism *via* the production of carnitine under stress conditions. Glutamic acid was the highest among all the free amino acids as shown in Table 3. Proline, which is known to function as a metal chelator, substance for antioxidant defense, and signal transmitter under stress conditions, was not increased along with stress treatment. Previous literatures (Hayat et al., 2012; Liang et al., 2013; Ben Rejeb et al., 2014; Meena et al., 2019) indicated that a stressful environment usually resulted in an overproduction of proline in cells in order to maintain osmotic balance, stabilize membranes, and keep ROS concentration within normal

ranges. However, decrease in proline level in the CBZ + NaOCl group was observed suggesting that proline metabolism may not be involved in the antioxidant systems of *T. obliquus* KNUA061. From our results, it is unclear whether the free amino acid regulation was involved in the redox mechanisms of the microalga to cope with CBZ and/or NaOCl stress conditions. Nevertheless, the high essential amino acid contents would enhance the value of microalgal biomass towards an alternative feedstock for nutrient dietary supplements.

IV is defined as the amount of iodine in a specific biodiesel and it is related to the number of double bonds that affect the DU, OS, and cold flow of biodiesel (Islam et al., 2013.; Cho et al., 2019). CN is also an important parameter that reflects engine performance, the generation of nitrous oxide, and the combustion of biodiesel (Islam et al., 2013.; Cho et al., 2019). Although the biodiesel properties of strain KNUA061 showed slightly higher values of IV (138–147) and lower values of CN (40–45) than the standard values (IV: ≤120; CN: ≥47), it is still expected that the biodiesel produced from *T. obliquus* KNUA061 could be used as a blending resource for transportation fuels since biodiesel blends with petrodiesel have been widely used as automotive fuels worldwide (Kousoulidou et al., 2010; Salvi and Panwar 2012; Du et al., 2018). However, further studies are needed to promote biodiesel properties by changing the cultivation conditions and other biotechnological techniques.

The ultimate analysis results showed that the HHVs (22.3–23.1) of strain KNUA061 biomass were higher than those of the terrestrial energy crops (17.0–20.0 MJ kg^{-1}) (Ross et al., 2008). Since fine particulate matters have become a national concern in Korea, a couple of coal-burning power stations have already converted to biomass-burning stations and many old coal-powdered plants are considering this move in the near future (Yang 2016; Um and Kang 2019). Hence, microalgal pellet made of mass-cultivated microalgae biomass could be an excellent mixed combustion biofuel for these coal power stations.

Many researches on microalgae-mediated bioremediation of pharmaceuticals have recently been reported (Peng et al., 2014; Matamoros et al., 2016b; Escapa et al., 2017; Xiong et al., 2017; Xiong et al., 2018). Also, a number of studies (Supplementary Table S8) have demonstrated biotransformation of CBZ by using a variety of microalgal species, but their removal rates were relatively lower than those by the advanced oxidation treatment methods (Supplementary Table S1). However, microalgal bioremediation of CBZ has gained growing scientific interest due to its many advantages such as carbon fixation *via* photosynthesis and low operational costs, and acclimatization of nitrogen and phosphorus into algal biomass over other means (Cai et al., 2013; Gonçalves et al., 2017; Xiong et al., 2018; Koul et al., 2022). Furthermore, this process is considered economically and environmentally sustainable because the resulting microalgal biomass after remediation can be reutilized as nutraceuticals, cosmetics, fertilizers, aquaculture and animal feeds, and biofuels.

Recent works by Özengin and Elmaci (2016), Chen et al. (2018), Casierra-Martinez et al. (2020), and Xie et al. (2022) demonstrated higher CBZ removal efficiencies in constructed wetlands (CWs) than those in conventional wastewater plants. Therefore, it is hoped that integration of CWs with microalgal culturing ponds would facilitate more efficient and environmentally friendly remediation of CBZ in the future.

5 Concluding remarks

This study demonstrated the possibility of biological removal of CBZ by using an indigenous microalga, *T. obliquus* KNUA061. It was determined that efficiency of the microalgal biodegradation process could be accelerated by applying an appropriate concentration of NaOCl without inhibiting cell growth. This increase may account for the redox-defence system in microalgal cells that is activated to remove ROS generated by NaOCl. Increased levels of antioxidant enzymes and lipid oxidation were also observed when compared to those of the control. Thus, *T. obliquus* KNUA061 could be a promising candidate for microalgae-mediated bioremediation of CBZ. In addition, it was also confirmed that the resulting biomass was rich in essential amino acids and PUFAs and its HHV is higher than terrestrial energy crops. Therefore, the biomass has a great potential as biodiesel blend and mixed combustion biofuel as well as dietary supplement. Ellis, 2008, Loos et al., 2018.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

Author contributions

J-MD was involved in the conceptualization, laboratory work, data analysis, drafting, and editing of the manuscript; H-TY was involved in experimental design and data curation; JWH was involved in editing of the manuscript; G-SD was involved in laboratory work; H-SY was involved in the conceptualization and editing of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenrg.2022.1004613/full#supplementary-material

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