Supporting Information

Effects of $Ti_3C_2T_x$ (MXene) on growth, oxidative stress, and metabolism of *Microcystis aeruginosa*

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Material and Methods

Hydrogen Peroxide (Catalase, CAT) Assay Kit method

When conducting experiments with the Catalase Assay Kit, sample preparation initially involves collecting cell, tissue, or blood samples and processing them with appropriate lysis or homogenization solutions. For cellular samples, they are washed with pre-chilled PBS or physiological saline and then homogenized, followed by centrifugation to obtain the supernatant as the sample for testing. Tissue samples are homogenized using pre-chilled PBS in an ice bath, and the supernatant is collected after centrifugation. The preparation of plasma or red blood cell samples may require collection in an anticoagulant tube, followed by centrifugation and dilution. The preparation of the assay kit includes the formulation of a 250 mM hydrogen peroxide solution, which typically involves diluting the approximately 1M hydrogen peroxide provided in the kit with the assay buffer in a 100-fold dilution. Subsequently, a 5 mM hydrogen peroxide solution is prepared based on the actual measured concentration of hydrogen peroxide, and the color development solution is made by dissolving the color development substrate in an ice bath and mixing it with the catalase to form the color development working solution. The standard curve is established by taking hydrogen peroxide standard solutions of various concentrations, adding the color development working solution, incubating at 25°C for 15 minutes, and then measuring the absorbance at 520 nm to establish the relationship between absorbance and hydrogen peroxide concentration. During the sample assay, the test sample is mixed with the hydrogen peroxide assay buffer and a 250 mM hydrogen peroxide solution, and

incubated for 1-5 minutes. After the reaction, the hydrogen peroxide reaction termination solution is added to halt the reaction. A specific volume of the reaction mixture is then transferred to a 96-well plate, the color development solution is added, and the mixture is incubated again before measuring the absorbance at 520 nm. The catalase activity is calculated based on the standard curve by comparing the absorbance difference between the sample well and the blank control well to determine the amount of hydrogen peroxide consumed in the sample, thereby calculating the catalase activity in the sample. For cellular or tissue samples, the catalase activity is converted to units per mg of protein based on the protein concentration and the dilution factor. This process requires precise operation to ensure the accuracy and reproducibility of the results.

Malondialdehyde (MDA) Assay Kit method

When utilizing a Lipid Peroxidation (MDA) Assay Kit for experimentation, sample preparation initially necessitates the collection of liquid samples such as plasma, serum, or urine, which can be directly employed for MDA measurement. For tissue or cellular samples, homogenization or lysis with an appropriate lysis solution is required, followed by centrifugation to separate the supernatant for subsequent analysis. In cases where the MDA concentration in the samples is high, dilution may be necessary. The assay kit preparation involves weighing a specific amount of TBA to prepare a 0.37% TBA storage solution, which must be fully dissolved before use, with the option to heat it to 70°C to facilitate dissolution. The MDA assay working solution is prepared by freshly formulating an appropriate volume based on the number of samples to be tested,

involving the mixture of TBA dilution, TBA storage solution, and antioxidants. For sample measurement, 0.1 ml of the homogenate, lysate, or appropriate solution such as PBS is used as a blank control, and different concentrations of standards are added for the construction of a standard curve. 0.1 ml of the sample is used for the assay, followed by the addition of 0.2 ml MDA assay working solution. After mixing, the mixture is heated for 15 minutes in a 100°C water bath or boiling water bath, taking care to prevent splashing. Post-heating, the mixture is cooled to room temperature, centrifuged at 1000g for 10 minutes. 200 μl of the supernatant is transferred to a 96-well plate, and the absorbance is measured at 532 nm using a spectrophotometer. If measuring at 532 nm is not feasible, the absorbance can also be measured between 530-540 nm. The calculation of MDA content involves determining the molar concentration of MDA in the samples based on the standard curve. For cellular or tissue samples, once the MDA content in the sample solution is calculated, it can be expressed in terms of the initial sample's MDA content per unit weight of protein or tissue, such as μmol/mg protein or μmol/mg tissue. If the MDA concentration in the samples is low, more tissue or cells may be required, and dilution of the samples should be avoided if possible. If the MDA concentration is high, the concentrations of the standards can be increased to 100, 150, and 200 μM. These steps enable the accurate determination of MDA levels in samples, providing crucial data for the study of lipid peroxidation.

Superoxide Dismutase (SOD) Assay Kit method

When employing the Total Superoxide Dismutase (SOD) Assay Kit with NBT method for experiments, sample preparation initially involves the collection of cellular,

tissue, or blood samples, followed by treatment with appropriate lysis or homogenization solutions. For cellular samples, they are washed with pre-chilled PBS or physiological saline and then homogenized, after which centrifugation is performed to obtain the supernatant for subsequent analysis. Tissue samples are homogenized using pre-chilled PBS in an ice bath, and the supernatant is collected after centrifugation. The preparation of plasma or red blood cell samples may require collection in an anticoagulant tube, followed by centrifugation and dilution. The assay kit preparation includes the formulation of the NBT/enzyme working solution, which involves mixing SOD assay buffer, NBT, and enzyme solution. Additionally, the reaction initiation working solution is prepared, typically by diluting the reaction initiator (40X) with SOD assay buffer in a specified ratio. During the sample assay phase, the test samples are mixed with the NBT/enzyme working solution, followed by the addition of the reaction initiation working solution and incubation at 37°C for 30 minutes. Subsequently, the absorbance is measured at 560 nm to evaluate SOD activity. The calculation of SOD activity is based on the percentage of inhibition, which requires comparing the absorbance difference between the sample well and the blank control well. Using the standard curve, the SOD activity units in the samples can be calculated. For cellular or tissue samples, the SOD activity units may need to be converted to U/g or U/mg protein, based on protein concentration and dilution factor. This process requires precise manipulation to ensure the accuracy and reproducibility of the results.

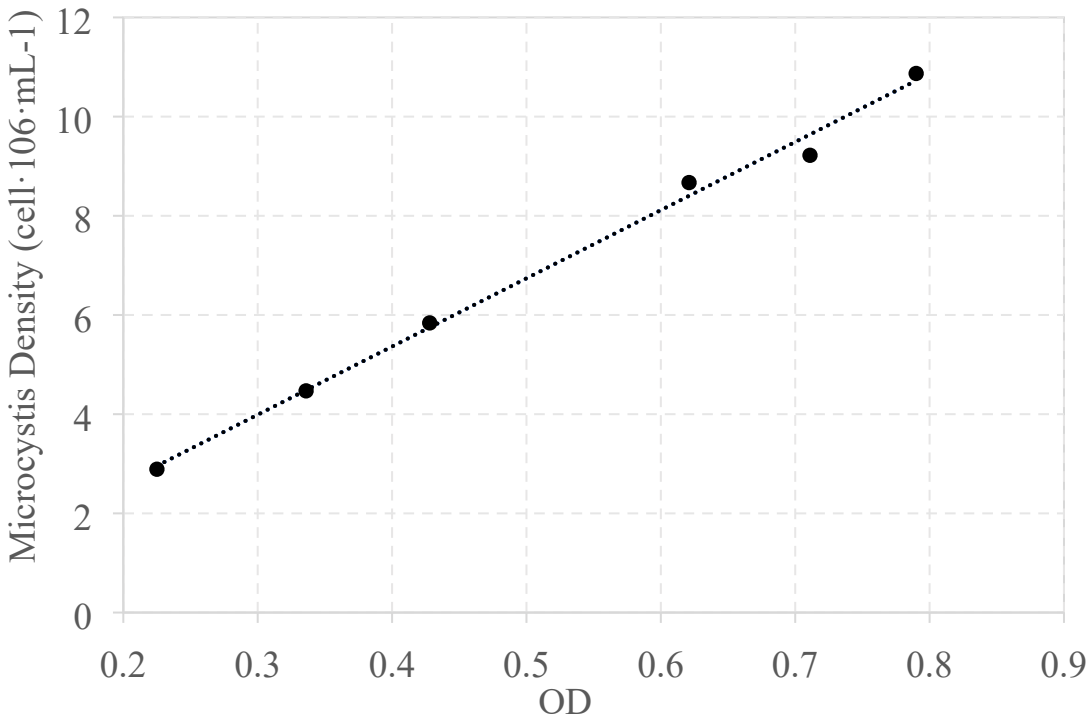

Figure S1. The linear relationship between OD_{680} values and *M. aeruginosa* cell density.

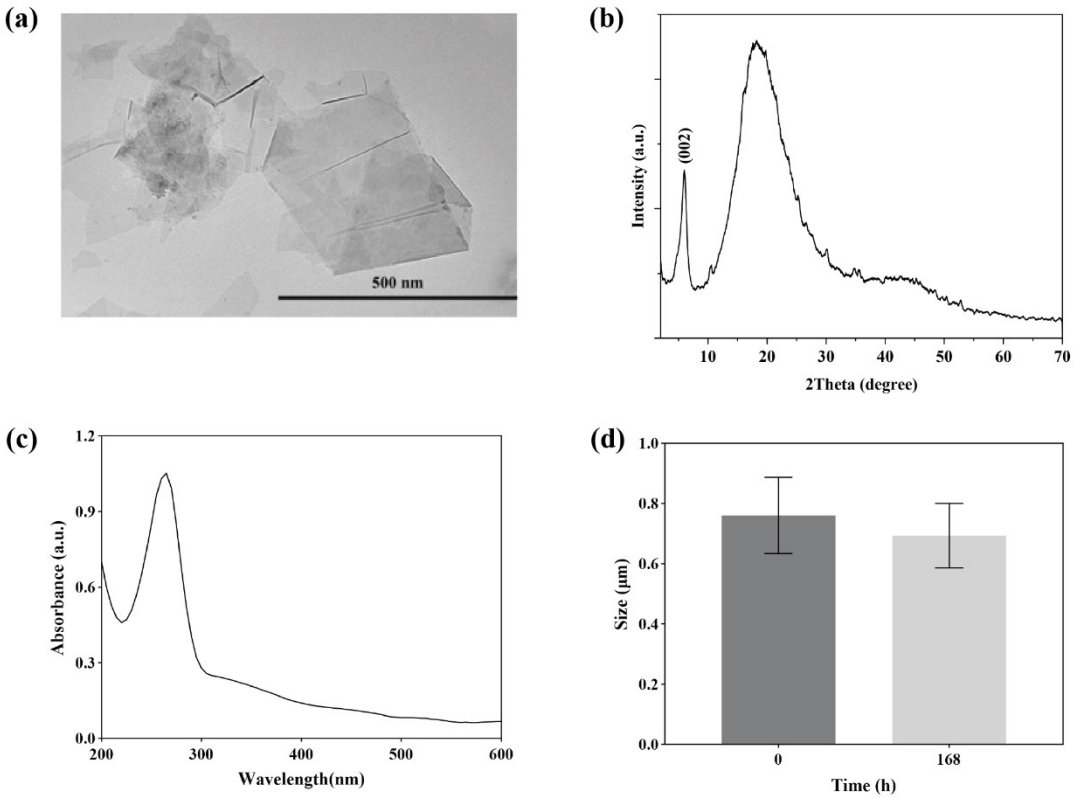

Figure S2. Physical and chemical characteristics of $Ti_3C_2T_x$. (a) Transmission electron microscopy image of $Ti_3C_2T_x$. (b) Typical XRD pattern of $Ti_3C_2T_x$. (c) UV absorption peak of $Ti_3C_2T_x$. (d) Hydrated size of $Ti_3C_2T_x$ in the exposure medium.

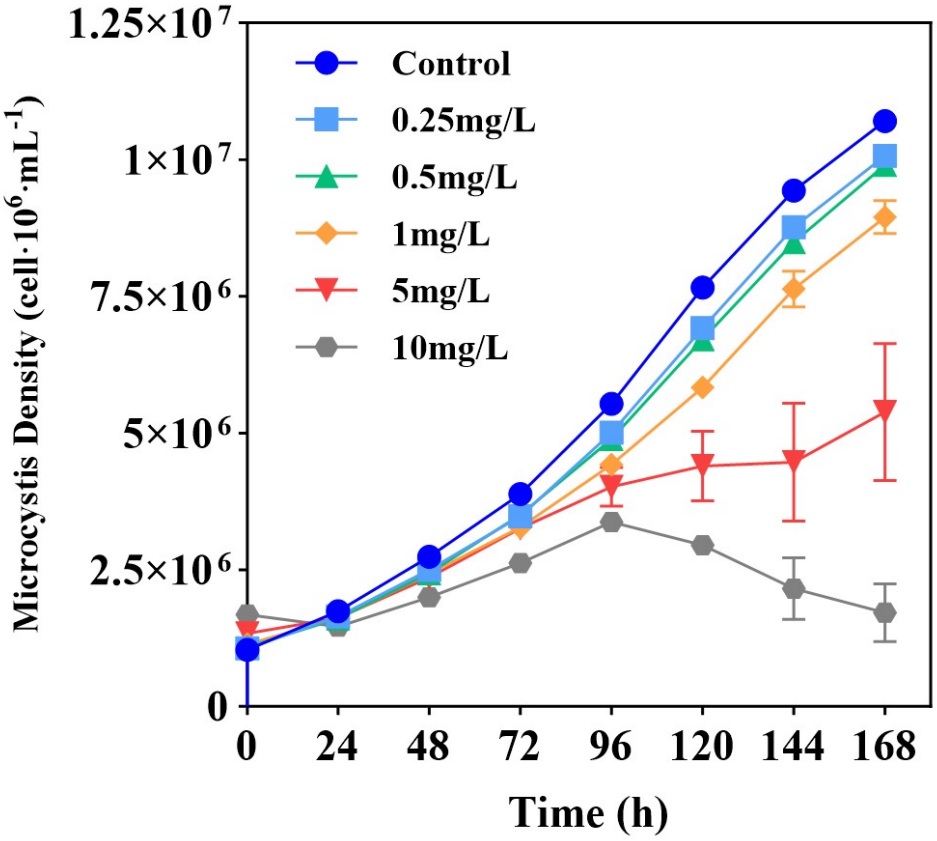

Figure S3. The impact of different concentrations of $Ti_3C_2T_x$ on the density of *M. aeruginosa* based on microscopic cell counting.

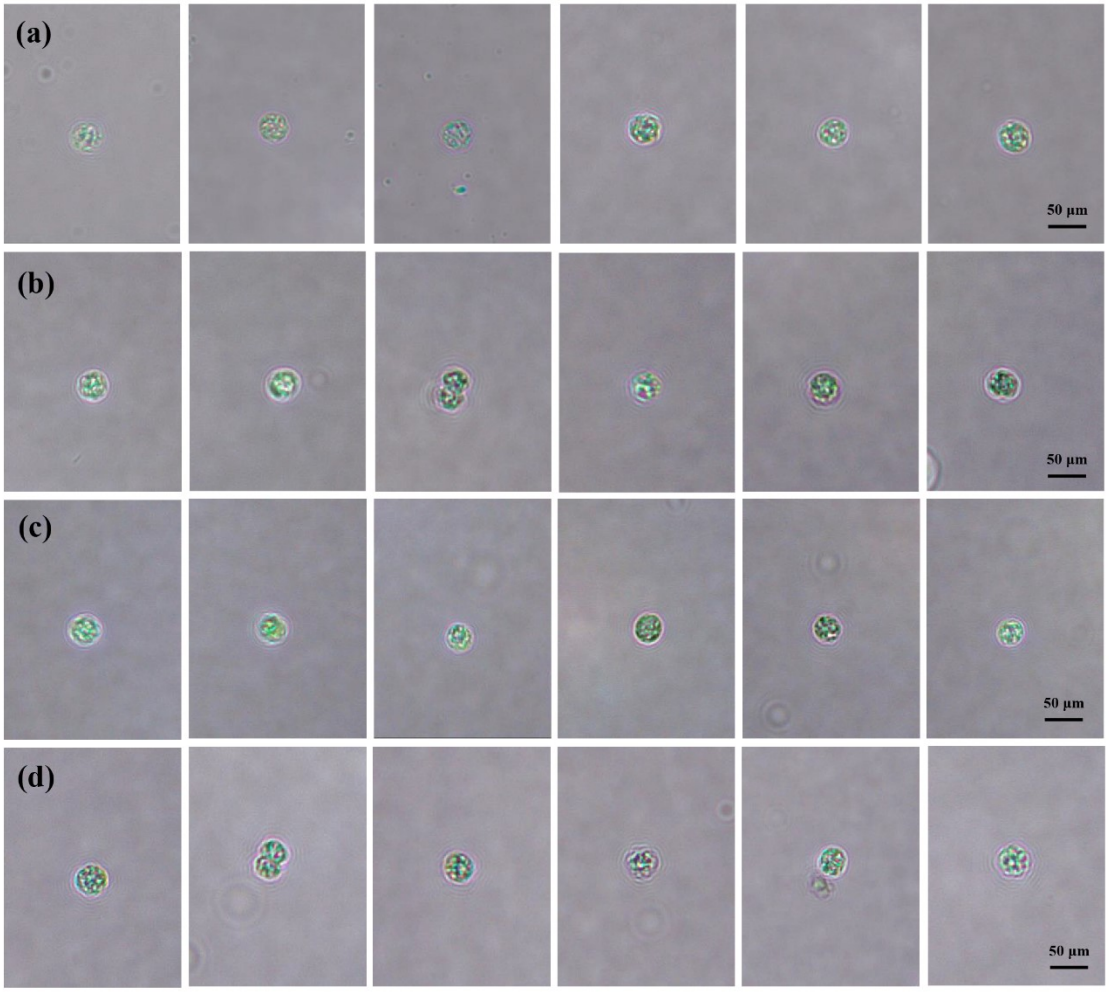

Figure S4. Optical microscope images of *M. aeruginosa* cells in each treatment group exposed to $Ti_3C_2T_x$. (a) Control group; (b) 1 mg/L of $Ti_3C_2T_x$; (c) 5 mg/L of $Ti_3C_2T_x$; (d) 10 mg/L of $Ti_3C_2T_x$.

Table S1. The composition of COMBO medium.

Composition of COMBO	
Compound	Concentration (g/L)
Seven major components	
$CaCl_2$ $2H_2O$	36.76
$MgSO_4$ $7H_2O$	36.97
K_2HPO_4	8.71
$NaNO_3$	85.01
$NaHCO_3$	12.60
Na_2SiO_3 $9H_2O$	28.42
H_3BO_3	24
KCl	7.45
Alage trace elements (ATE)	
Na_2EDTA $2H_2O$	4.36
$FeCl_3$ H_2O	1.0
$MnCl_2$ $4H_2O$	0.18
$CuSO_4$ $5H_2O$	0.001
$ZnSO_4$ $7H_2O$	0.022
$CoCl_2$ 6H2O	0.012
$NaMoO_4$ $2H_2O$	0.022
H_2SeO_3	0.0016
Na_3VO_4	0.0018
Vitamins (VIM)	
B_{12}	0.00055
Biotin	0.0005
Thiamin	0.1

Preparation Instructions: During the preparation of 1 liter of culture medium, take 1 mL of each from the "Seven major components"; homogenize the "Algal trace elements (ATE)" and take 1 mL; homogenize the "Vitamins (VIM)" and take 1 mL. Finally, make up to a final volume of 1 liter.

Table S2. Analysis of Variance (ANOVA).

	ANOVA table	SS (Sum of Squares)	DF (Degree of Freedom)	MS (Mean Square)	F (F-test)	P (p-value)
Algal OD	Concentration	10.724	5	2.1448	11.528	0.0001
	Time	38471.7824	7	5495.9689	29540	0.0001
	AxB	6.5118	35	0.1861	0	0.9999
	Error	360330.3678	149	2418.3246		
	Total Variation	398819.3861	196			
Inhibition rate	Treatment (between columns)	0.222064	3	0.000688	0.3178	0.8125
	Residual (wuthin columns)	0.01732	8	0.002165		
	Total	0.01939	11			
MDA	Treatment (between columns)	0.222064	3	0.000688	0.3178	0.8125
	Residual (wuthin columns)	0.01732	8	0.002165		
	Total	0.01939	11			
CAT	Treatment (between columns)	236.3	3	78.75	2.607	0.1239
	Residual (wuthin columns)	241.6	8	30.21		
	Total	477.9	11			
SOD	Treatment (between columns)	52.2	3	17.4	3.479	0.0704
	Residual (wuthin columns)	40.02	8	5.002		
	Total	92.22	11			
Photosynthetic efficiency	Treatment (between columns)	0.249	5	0.05	3.994	0.023
	Residual (wuthin columns)	0.15	12	0.012		
	Total	0.399	17			