Supporting Information

Analyzing the Biochemical Alteration of Green Algae During Chronic Exposure to Triclosan Based on Synchrotron-Based Fourier Transform Infrared Spectromicroscopy

Xiaying Xin,[a] Gordon Huang,[a,*] Chunjiang An,[b] Harold Weger,[c] Guanhui Cheng,[a] Jian Shen[a], Scott Rosendahl[c]

[a] Institute for Energy, Environment and Sustainable Communities, University of Regina, Regina, Canada S4S 0A2

[b] Department of Building, Civil and Environmental Engineering, Concordia University, Montreal, Canada H3G 1M8

[c] Department of Biology, University of Regina, Regina, Canada S4S 0A2

[d] Canadian Light Source, Saskatoon, Saskatchewan, Canada S7N 2 V3

*Corresponding author: Tel: +1-306-5854095; Fax: +1-306-5854855; E-mail: gordon.huang@uregina.ca

This SI file contains:

29 Pages

Page S-2 - S-5……..4 Narrative section

Page S-6 - S-7……..Figure and Table captions

Page S-8 - S-19……Figure S1 - Figure S11

Page S-20 - S-28……..Table S1- Table S6

Page S-29…….REFERENCES

EXPERIMENTAL SECTION

Determination of triclosan concentration. Culture solution was sampled at 0 h and 120 h from each treatment. All samples were centrifuged at 12,000 × g for 10 min. Triclosan in supernatant was concentrated by solid phase extraction[1] and triclosan in algae was extracted following Ding's method.[2] Triclosan was measured in both media and cells. Triclosan was quantified through Agilent 1260 liquid chromatograph equipped with a diode array detector (HPLC-DAD) (Santa Clara, CA, USA). A ZORBAZ XDB-C18 column (250×4.6 mm, 5 μm, Agilent) was used. The mobile phase was 70/30 mixture of acetonitrile/water with a flow rate of 0.8 mL/min. The detection wavelength was at 214 nm. The sample injection volume was 50 μL. The column temperature was maintained at 40 ˚C.[3]

RESULTS AND DISCUSSION

Measured triclosan concentrations. The concentration of triclosan in 32 treatments were measured and the results are shown in Figure S3. The final average concentrations of triclosan in the treatments with nominal concentrations of 113 μg/L were in the range of 74.01 to 91.17 %, higher than 70 % of the nominal concentrations on 120h. It indicated that triclosan was relatively stable during the exposure period. The loss of triclosan could be attributed to photodegradation, bio-adsorption, bioaccumulation and biodegradation by *Chlorococcum* sp..

Responses to the variations of environmental factors. In this study, statistical analysis considering six responses, cell density, Lipid (C-H)/Amide II, Lipid (C=O)/Amide II, Amide I/Amide II, Phosphoryl (P=O)/Amide II, and Saccharides (C-O-C)/Amide II, were conducted to evaluate the toxicity of triclosan. As shown in Figure S4, there were no significant factors for cell density in the control experiments after a 120-h exposure to triclosan. For Lipid (C-H)/Amide II, the significant factors

were phosphorus concentration and temperature above the Bonferroni limit. For Lipid (C=O)/Amide II, the significant factors were phosphorus concentration, temperature, salinity * phosphorus concentration, nitrogen concentration * phosphorus concentration, and nitrogen concentration. For Amide I/Amide II, the significant factor was phosphorus concentration above the Bonferroni limit. For Phosphoryl (P=O)/Amide II, the significant factor was nitrogen concentration above the Bonferroni limit. For Saccharides (C-O-C)/Amide II, the significant factors were temperature, phosphorus concentration, temperature * nitrogen concentration * phosphorus concentration and temperature * phosphorus concentration. These results showed phosphorus concentration and temperature were two main factors affecting biochemical components of green microalga in the absence of triclosan. Nitrogen concentration was the main factor for nucleic acids.

Analysis of interactions among environmental factors. Two-level interactions. Temperature * phosphorus concentration was above the Bonferroni limit for Lipid (C-H) /Amide II, Amide I /Amide II, and Saccharides (C-O-C)/Amide II, indicating its key role in altering biochemical components (Figure S11 A_1, A_2 and A_3). In general, temperature had the most dominant influence on phosphorus-inhibited growth rate of algae.[4] However, our finding showed the increase of temperature to the optimum did not cause the increase in all cellular components no matter how much phosphorus there was.

Temperature * NaCl concentration was above the Bonferroni limit for Lipid (C=O) /Amide II, Amide I /Amide II, and phosphodiester (P=O)/Amide II (Figure S11 B_1, B_2 and B_3). The lipid content of *Chlorococcum sp.* rose with the increase of salinity but decreased with the increase of temperature. It is reported that *Schizochytrium limacinum* OUC88 had the highest lipid content at salinities of 0.9–

3.6% (w/v) and temperature range of 16–30 °C.[5] The responsive action to the interaction of temperature and salinity was to regulate the degree of fatty acid unsaturation to maintain the normal membrane lipid physical state.

pH * nitrogen concentration was above the Bonferroni limit for Amide I /Amide II and saccharides (C-O-C)/Amide II (Figure S11 C_1 and C_2). In general, increased nitrogen leads increased proteins, but our study showed the opposite situation (Table S4). Meanwhile, it is reported that low pH enriched protein fraction,[6] but protein content at low pH was lower than that at high pH in our study. This interaction also had negative effects on polysaccharides. It consists with Chen's study, in which the carbohydrates in wet algal biomass could be converted to soluble sugars via fermentation at low pH.[6]

pH * NaCl concentration was above the Bonferroni limit for phosphodiester (P=O)/Amide II and saccharides (C-O-C)/Amide II (Figure S11 D_1 and D_2). To date, few studies have examined such an interaction working on phosphodiester-related components and carbohydrates. Our result showed the connections between them.

Three- or higher-level interactions. Two-way interactions may be affected by third variable, such that the interaction varies over third variable. This reasoning may also be extended to further high-order interactions. Temperature * pH * NaCl concentration was significant three-level interaction for four specific responses (Table S4), indicating its most important role among all high-order interactions. Temperature * pH * NaCl concentration was the only significant factor above the Bonferroni limit for cell density. It is above the Bonferroni limit for the other three responses and negatively affected them. Such interaction played an important role in the variation of lipids, and proteins, especially cell growth.

Temperature * NaCl concentration * phosphorus concentration, pH * NaCl concentration * phosphorus concentration, and temperature * nitrogen concentration * phosphorus concentration were significant three-level interactions for three specific responses. These three interactions played significant roles in lipid-related responses. pH * NaCl concentration * nitrogen concentration, temperature * pH * phosphorus concentration, pH * nitrogen concentration * phosphorus concentration, and NaCl concentration * nitrogen concentration * phosphorus concentration were significant three-level interactions for two specific responses. All these three interactions had a significant effect on saccharides (C-O-C)/Amide II, indicating that saccharides were more sensitive to higher level interactions. Furthermore, temperature * NaCl concentration * nitrogen concentration were the only significant three-level interaction for lipid (C=O)/Amide II, but it is above the Bonferroni limit, indicating its important role in lipid variation.

There were some other significant higher-order interactions, which played more important roles in the long term than that in the short term. For example, temperature * pH * NaCl concentration * nitrogen concentration, and temperature * pH * nitrogen concentration * phosphorus concentration were significant for three specific responses. Temperature * NaCl concentration * nitrogen concentration * phosphorus concentration were significant for both lipid (C-H)/Amide II and saccharides (C-O-C)/Amide II. Temperature * pH * NaCl concentration * phosphorus concentration and pH * NaCl concentration * nitrogen concentration * phosphorus concentration were significant for lipid (C-H)/Amide II. Temperature * pH * NaCl concentration * nitrogen concentration * phosphorus concentration was a significant five-level interaction for phosphodiester (P=O)/Amide II.

List of figures and tables

Figure S1. The microscopic view of unicellular algae of *Chlorococcum* sp..

Figure S2. (A) **(**B) The Canadian light source, (C) Bruker Vertex 70v Interferometer / Hyperion 3000 IR Microscope

Figure S3. Triclosan concentrations at 120 h for all treatments.

Figure S4. Half-normal probability plot of the effects for control experiments: (A) Cell density, (B) Lipid (C-H)/Amide II, (C) Lipid (C=O)/Amide II, (D) Amide I /Amide II, (E) Phosphoryl (P=O)/Amide II, (F) Saccharides (C-O-C)/Amide II.

Figure S5. Half-normal probability plot of the effects for triclosan exposure experiments: (A) Cell density, (B) Lipid (C-H)/Amide II, (C) Lipid (C=O)/Amide II, (D) Amide I /Amide II, (E) Phosphoryl (P=O)/Amide II, (F) Saccharides (C-O-C)/Amide II.

Figure S6. Normal plot of residuals for (A) Cell density, (B) Lipid (C-H)/Amide II, (C) Lipid (C=O)/Amide II, (D) Amide I /Amide II, (E) Phosphoryl (P=O)/Amide II, (F) Saccharides (C-O-C)/Amide II.

Figure S7. Average SR-FTIR absorption spectra from the toxicity assessment of triclosan on green microalga *Chlorococcum* sp..

Figure S8. Main effects on biophysiological responses under triclosan exposure.

Figure S9. Pareto chart of triclosan exposure experiments: (A) Cell density, (B) Lipid (C-H)/Amide II, (C) Lipid (C=O)/Amide II, (D) Amide I /Amide II, (E) Phosphoryl (P=O)/Amide II, (F) Saccharides (C-O-C)/Amide II.

Figure S10. SR-FTIR spectra from the toxicity assessment of triclosan on green microalga *Chlorococcum* sp.: (A) average SR-FTIR absorption spectra of Run 1, (B) the second derivative spectrum of SR-FTIR of Run 1 in the 3000-2800 cm-1 region,

(C) the second derivative spectrum of SR-FTIR of Run 1 in the 1724-1585 cm-1 region.

Figure S11. Remaining two-order interactions above the Bonferroni limit under triclosan exposure.

Table S1. Triclosan given with its molecular information, water solubility and application area.

Table S2. Independent variables of the 2^5 full factorial design.

Table S3. Experimental Design matrix.

Table S4. Significant factors of cell density and relative cellular components.

Table S5. The contribution of all of main effects and interactions in toxicity assessment.

Table S6. Wavenumber (cm^{-1}) and assignment of the major bands in the synchrotron-based infrared spectra of *Chlorococcum* sp. cells.

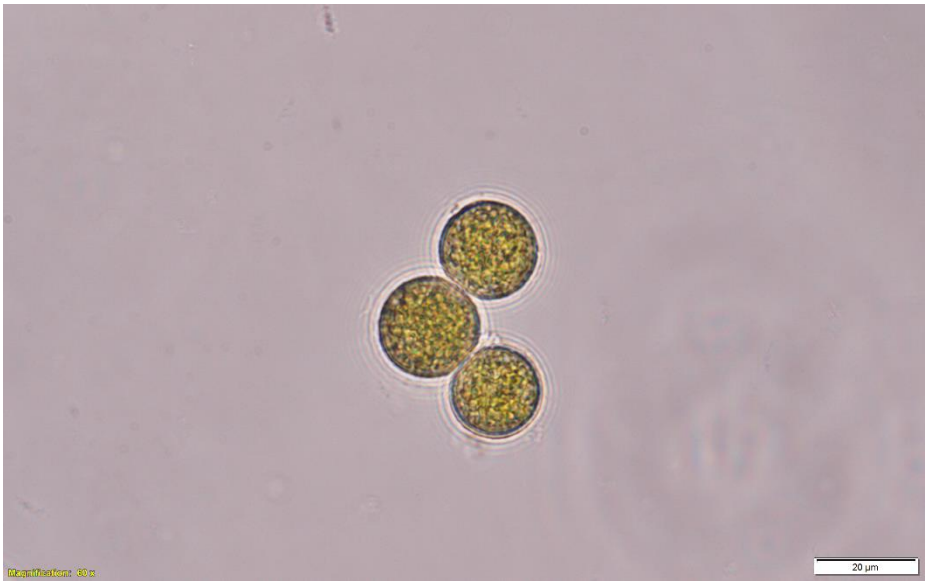
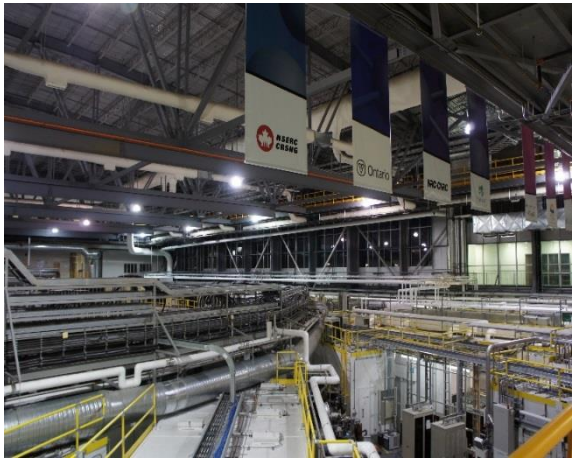


Figure S1. The microscopic view of unicellular algae of *Chlorococcum* sp..

(A)

(B)



(C)

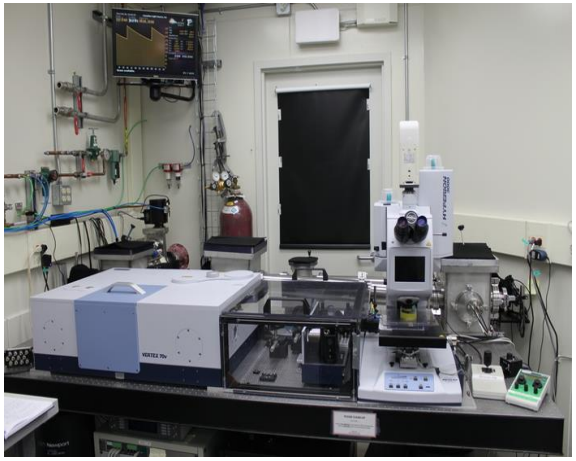


Figure S2. (A) **(**B) The Canadian light source, (C) Bruker Vertex 70v Interferometer / Hyperion 3000 IR Microscope.

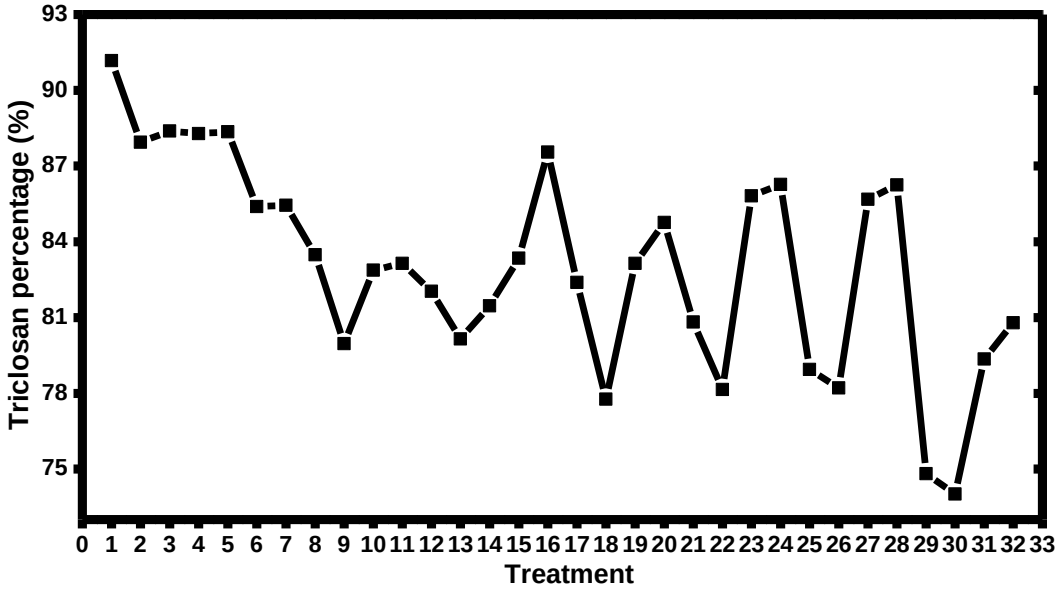
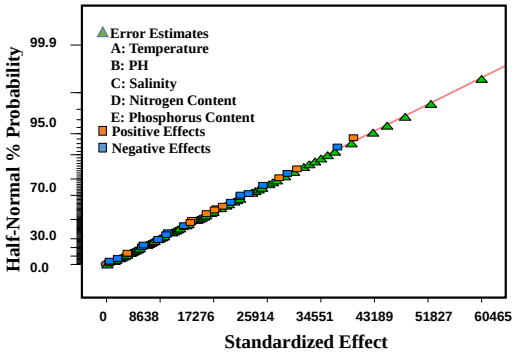
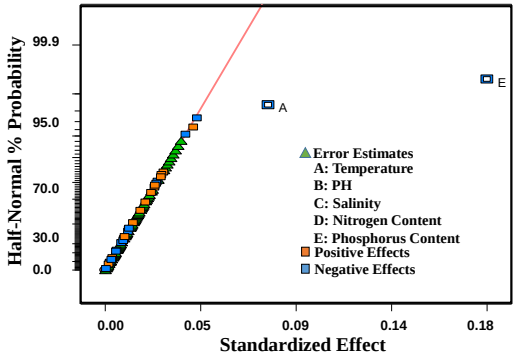


Figure S3. Triclosan concentrations at 120 h for all treatments.

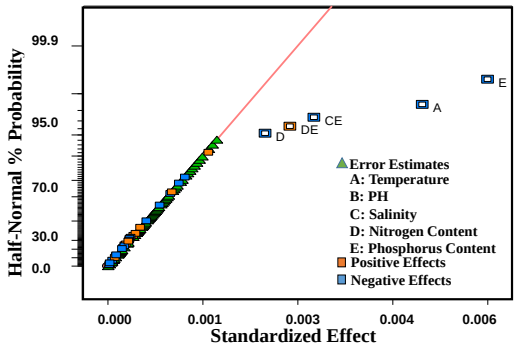
A: Cell density



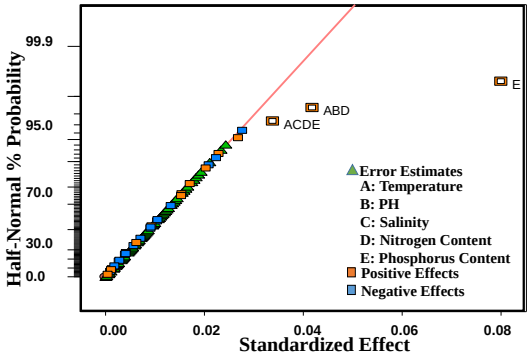
B: Lipid (C-H)/Amide II



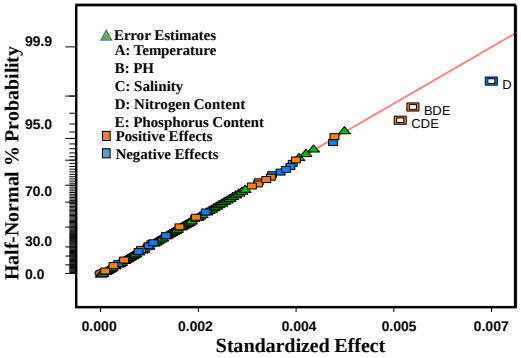
C: Lipid (C=O)/Amide II



D: Amide I /Amide II



E: Phosphoryl (P=O)/Amide II



F: Saccharides (C-O-C)/Amide II

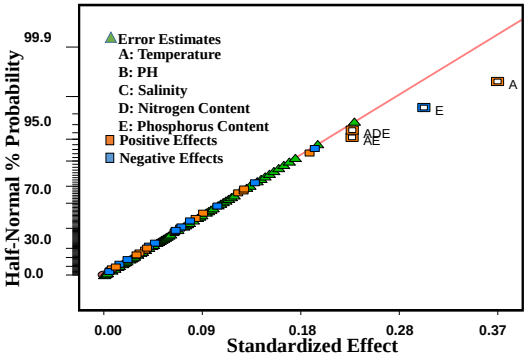
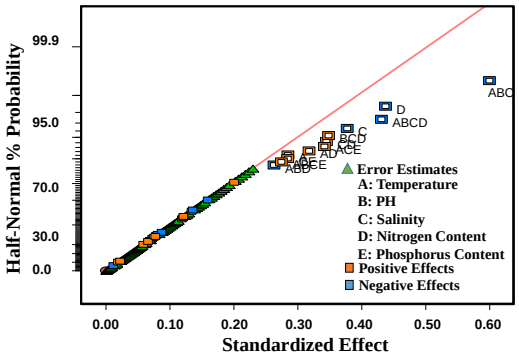
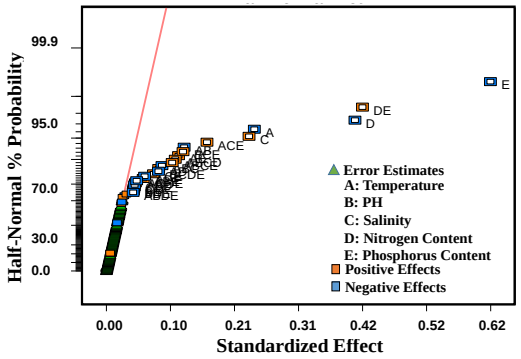


Figure S4. Half-normal probability plot of the effects for control experiments: (A) Cell density, (B) Lipid (C-H)/Amide II, (C) Lipid (C=O)/Amide II, (D) Amide I /Amide II, (E) Phosphoryl (P=O)/Amide II, (F) Saccharides (C-O-C)/Amide II.

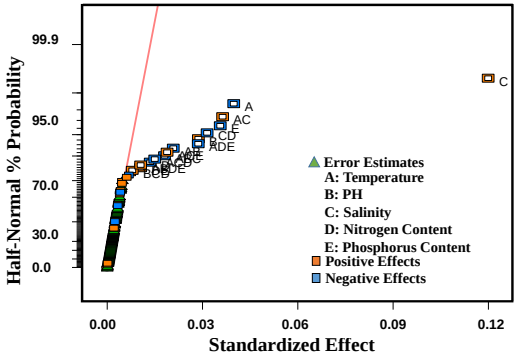
A: Cell density



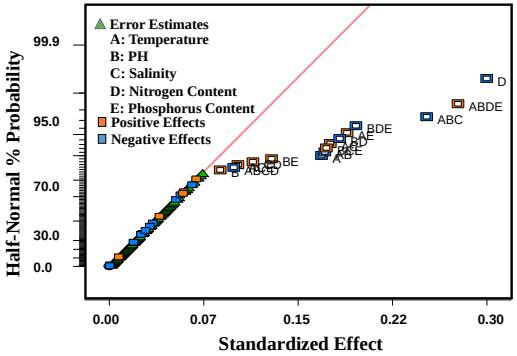
B: Lipid (C-H)/Amide II



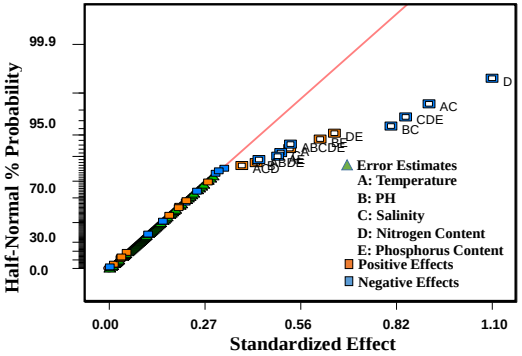
C: Lipid (C=O)/Amide II



D: Amide I /Amide II



E: Phosphoryl (P=O)/Amide II



F: Saccharides (C-O-C)/Amide II

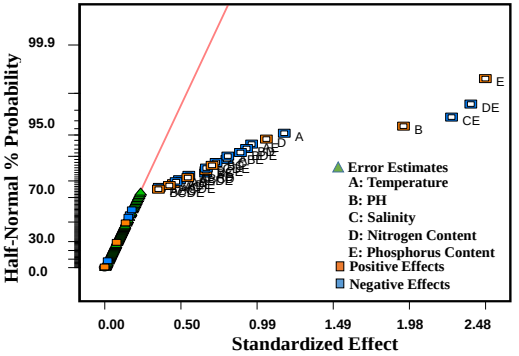


Figure S5. Half-normal probability plot of the effects for triclosan exposure experiments: (A) Cell density, (B) Lipid (C-H)/Amide II, (C) Lipid (C=O)/Amide II, (D) Amide I /Amide II, (E) Phosphoryl (P=O)/Amide II, (F) Saccharides (C-O-C)/Amide II.

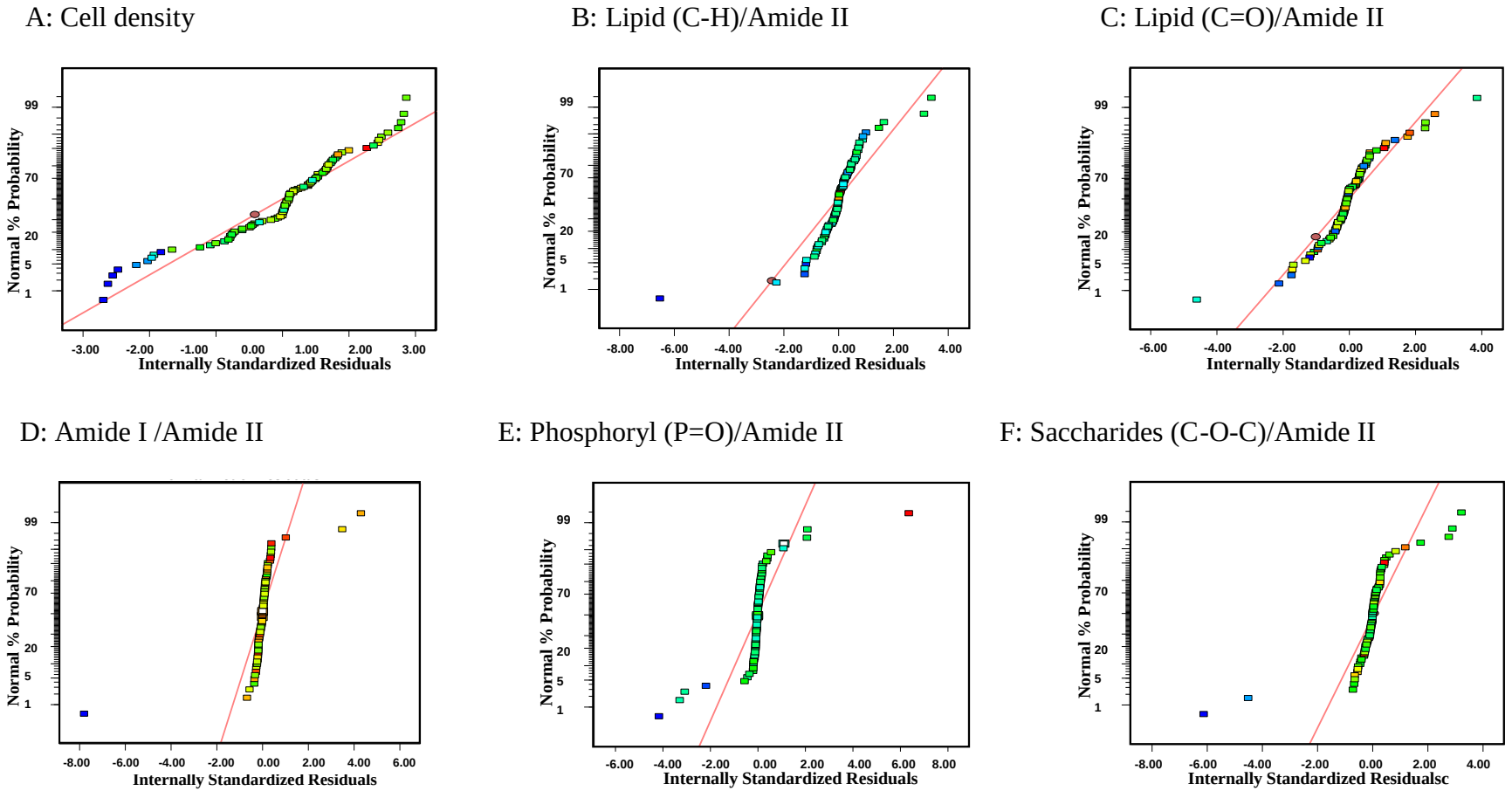
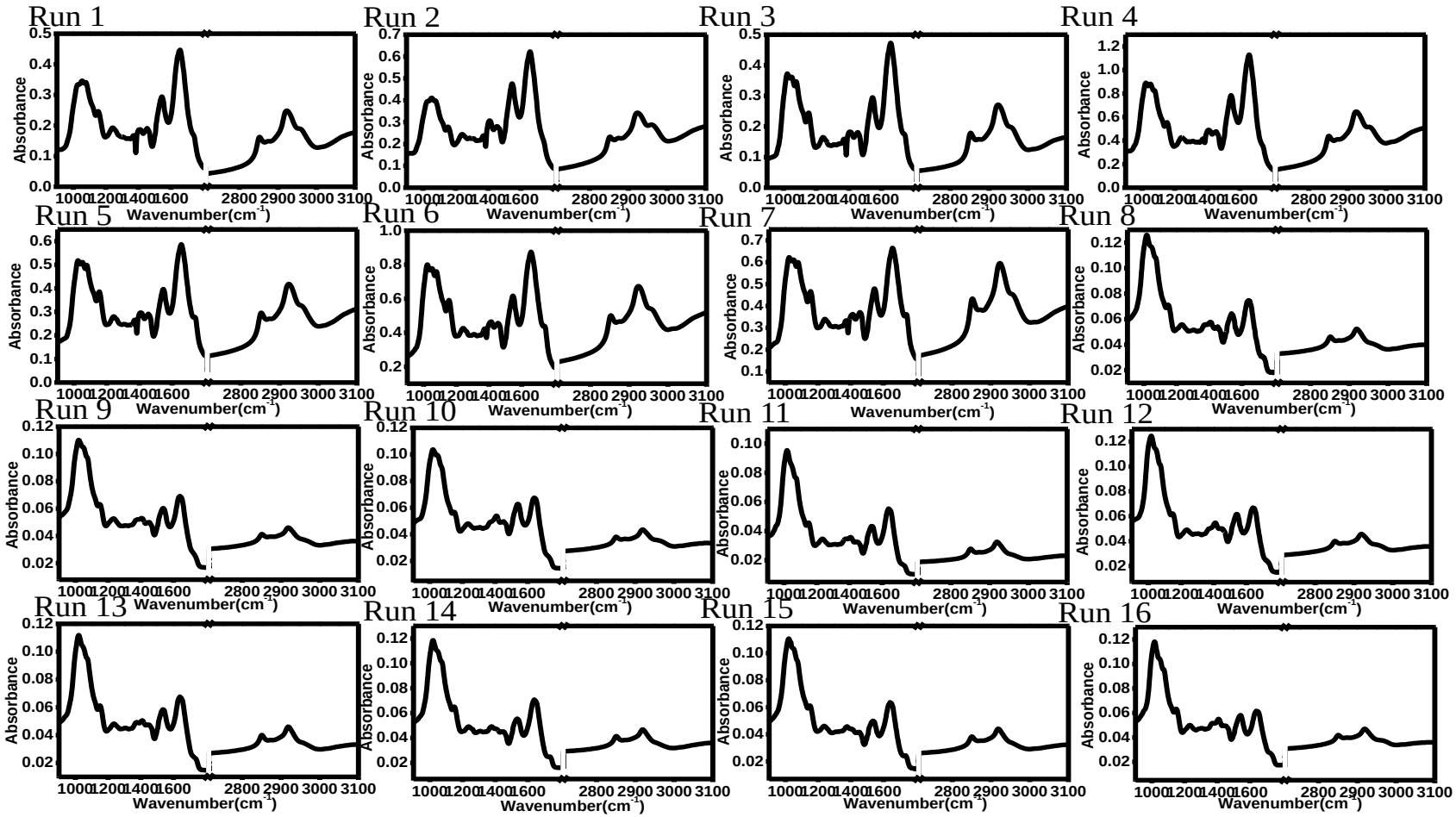


Figure S6. Normal plot of residuals for (A) Cell density, (B) Lipid (C-H)/Amide II, (C) Lipid (C=O)/Amide II, (D) Amide I /Amide II, (E) Phosphoryl (P=O)/Amide II, (F) Saccharides (C-O-C)/Amide II.


Run 1
Run 2
Run 3
Run 4
Run 5
Run 6
Run 7
Run 8
Run 9
Run 10
Run 11
Run 12
Run 13
Run 14
Run 15
Run 16
Absorbance
Wavenumber(cm-1)
1000 1200 1400 1600
2800 2900 3000 3100

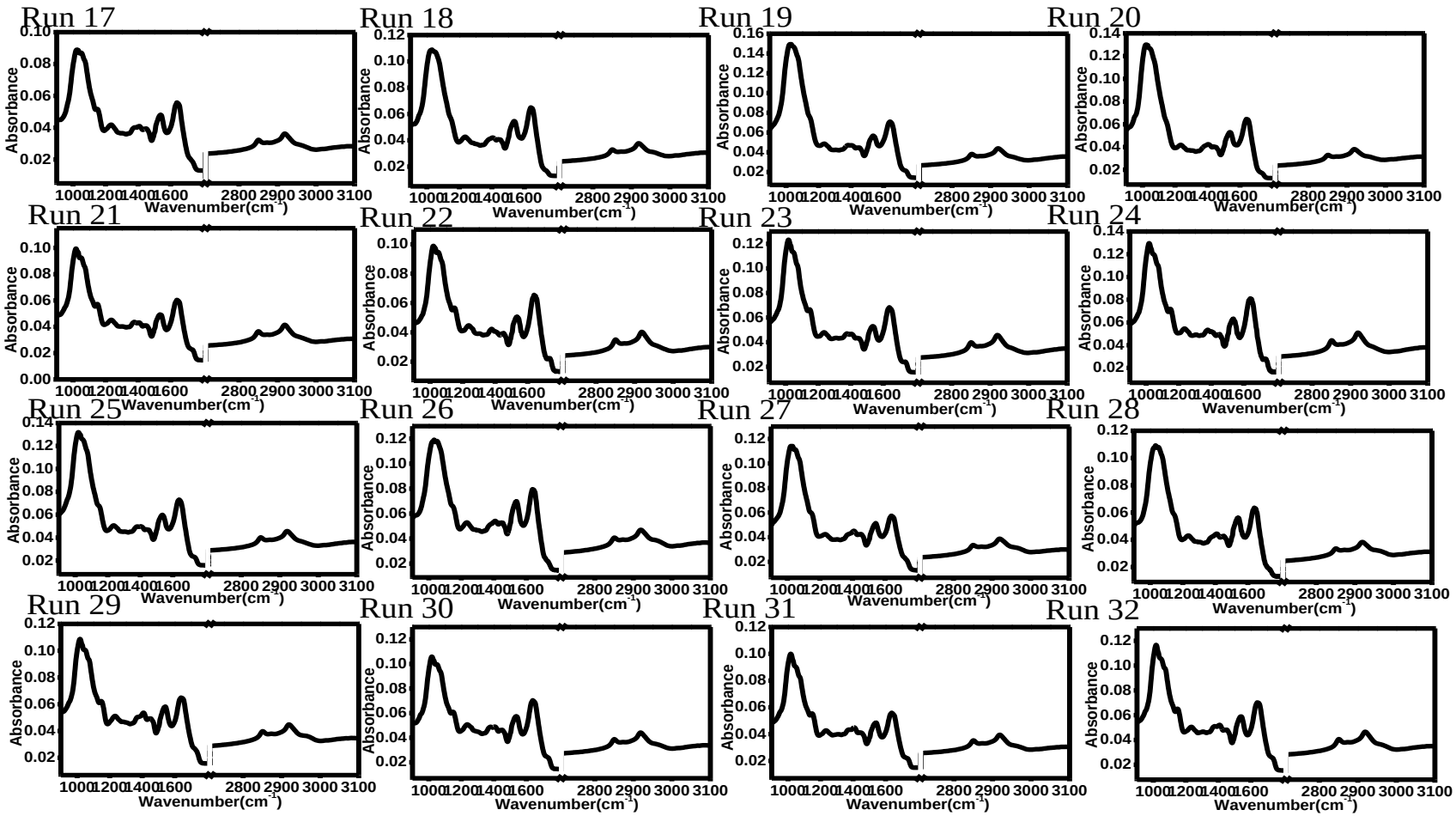


Figure S7. Average SR-FTIR absorption spectra from the toxicity assessment of triclosan on green microalga *Chlorococcum* sp..

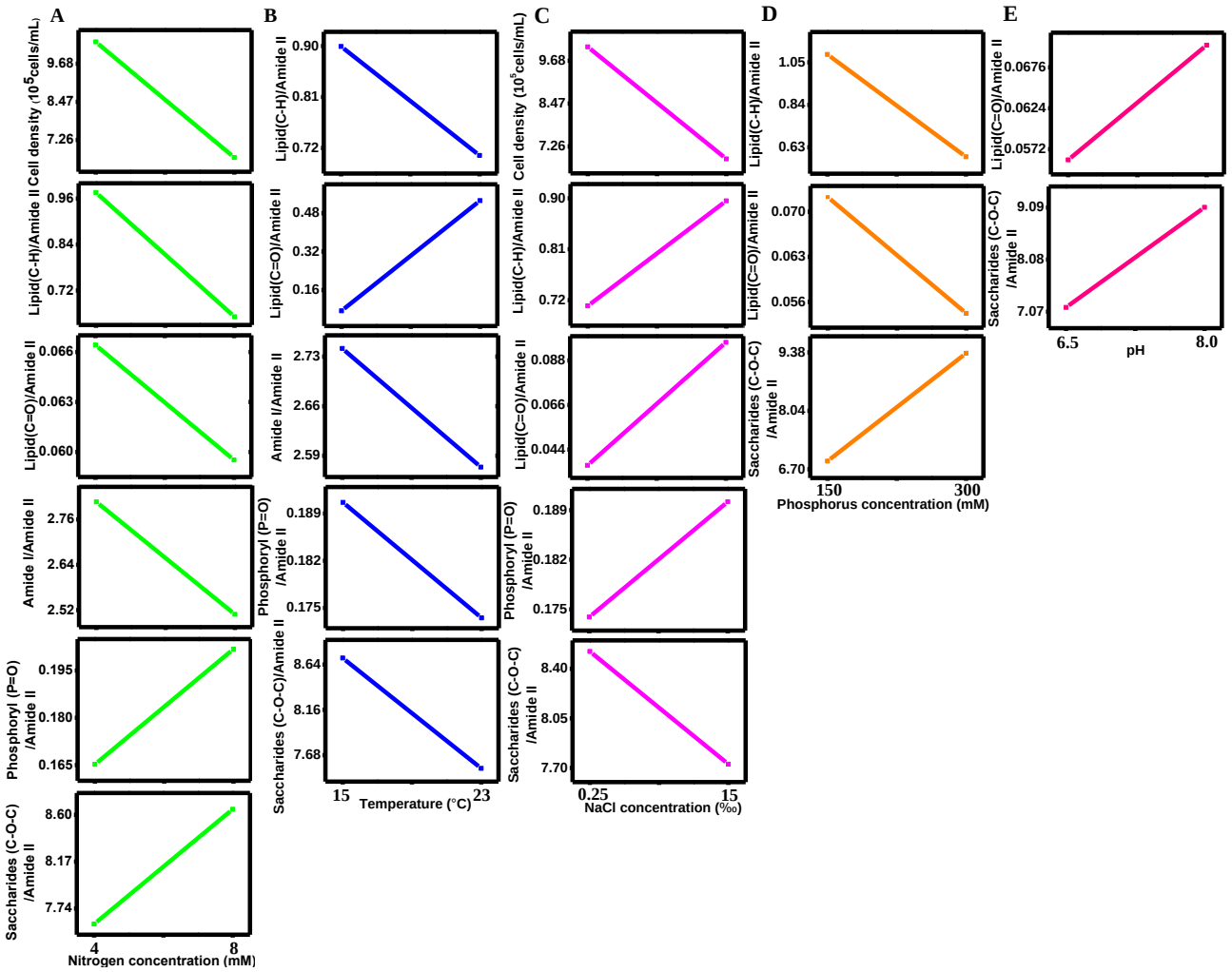
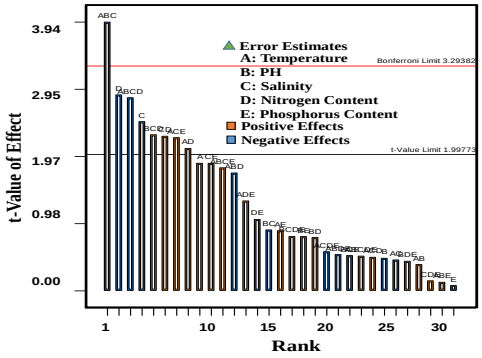
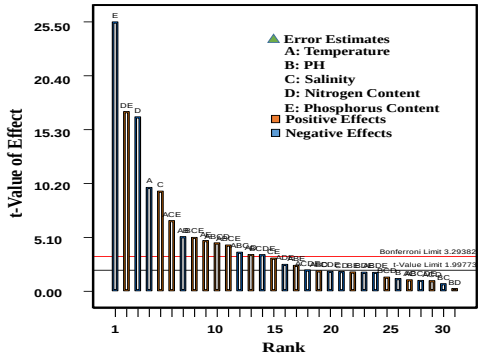


Figure S8. Main effects on biophysiological responses under triclosan exposure.

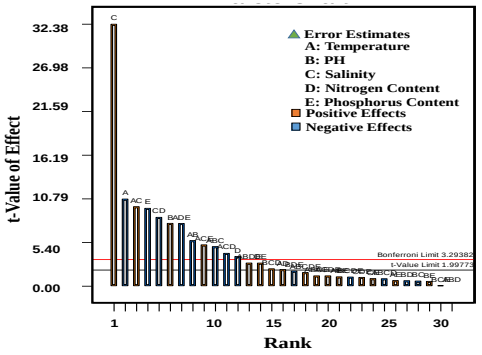
A: Cell density



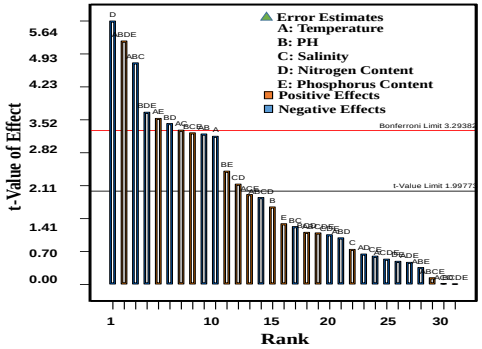
B: Lipid (C-H)/Amide II



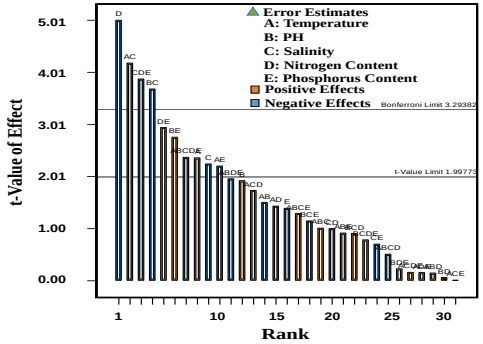
C: Lipid (C=O)/Amide II



D: Amide I /Amide II



E: Phosphoryl (P=O)/Amide II



F: Saccharides (C-O-C)/Amide II

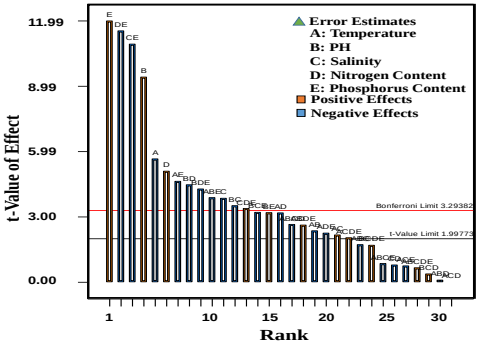


Figure S9. Pareto chart of triclosan exposure experiments: (A) Cell density, (B) Lipid (C-H)/Amide II, (C) Lipid (C=O)/Amide II, (D) Amide I /Amide II, (E) Phosphoryl (P=O)/Amide II, (F) Saccharides (C-O-C)/Amide II.

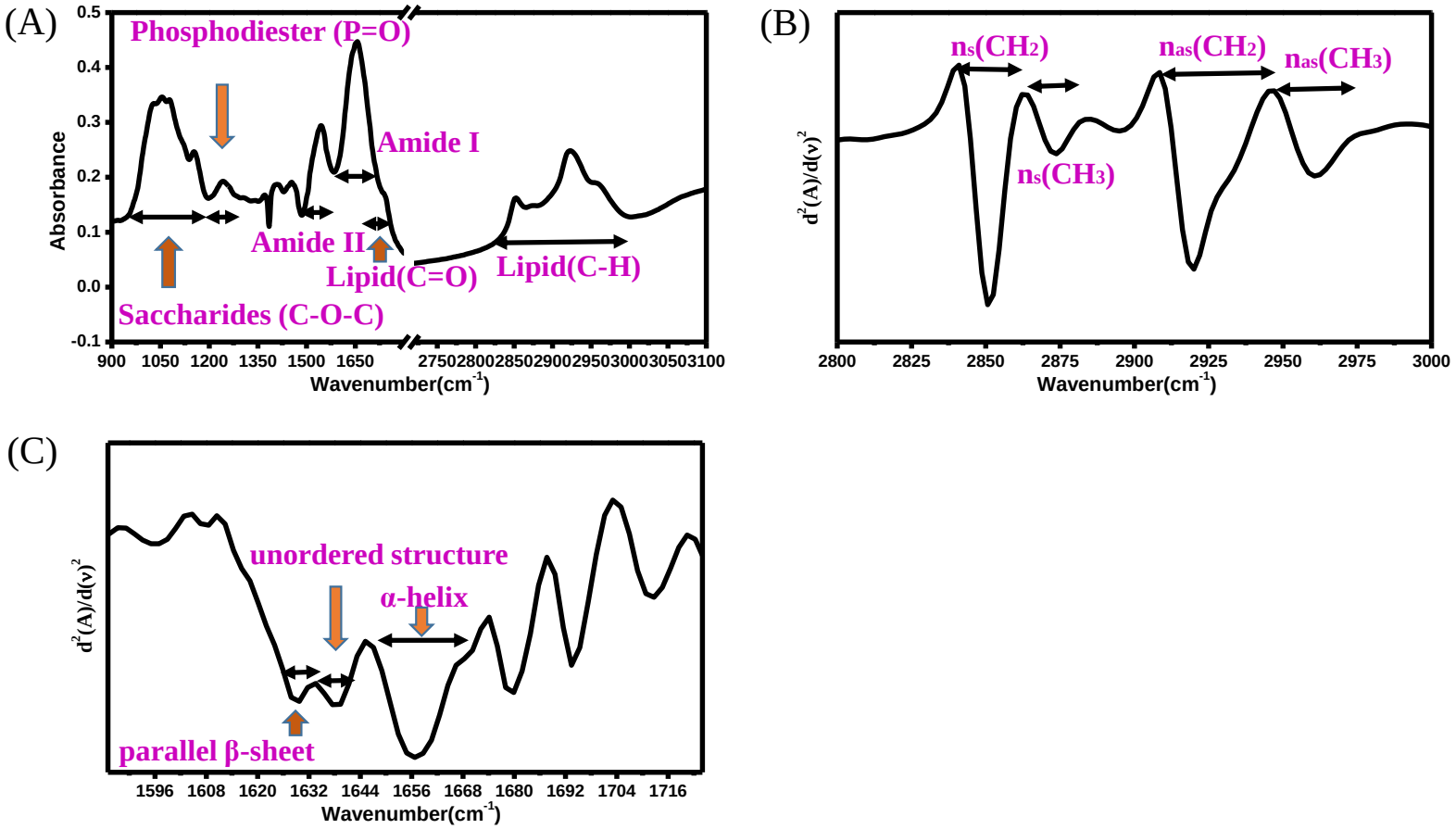


Figure S10. SR-FTIR spectra from the toxicity assessment of triclosan on green microalga *Chlorococcum* sp.: (A) average SR-FTIR absorption spectra of Run 1, (B) the second derivative spectrum of SR-FTIR of Run 1 in the 3000-2800 cm^{-1} region, (C) the second derivative spectrum of SR-FTIR of Run 1 in the 1724-1585 cm^{-1} region.

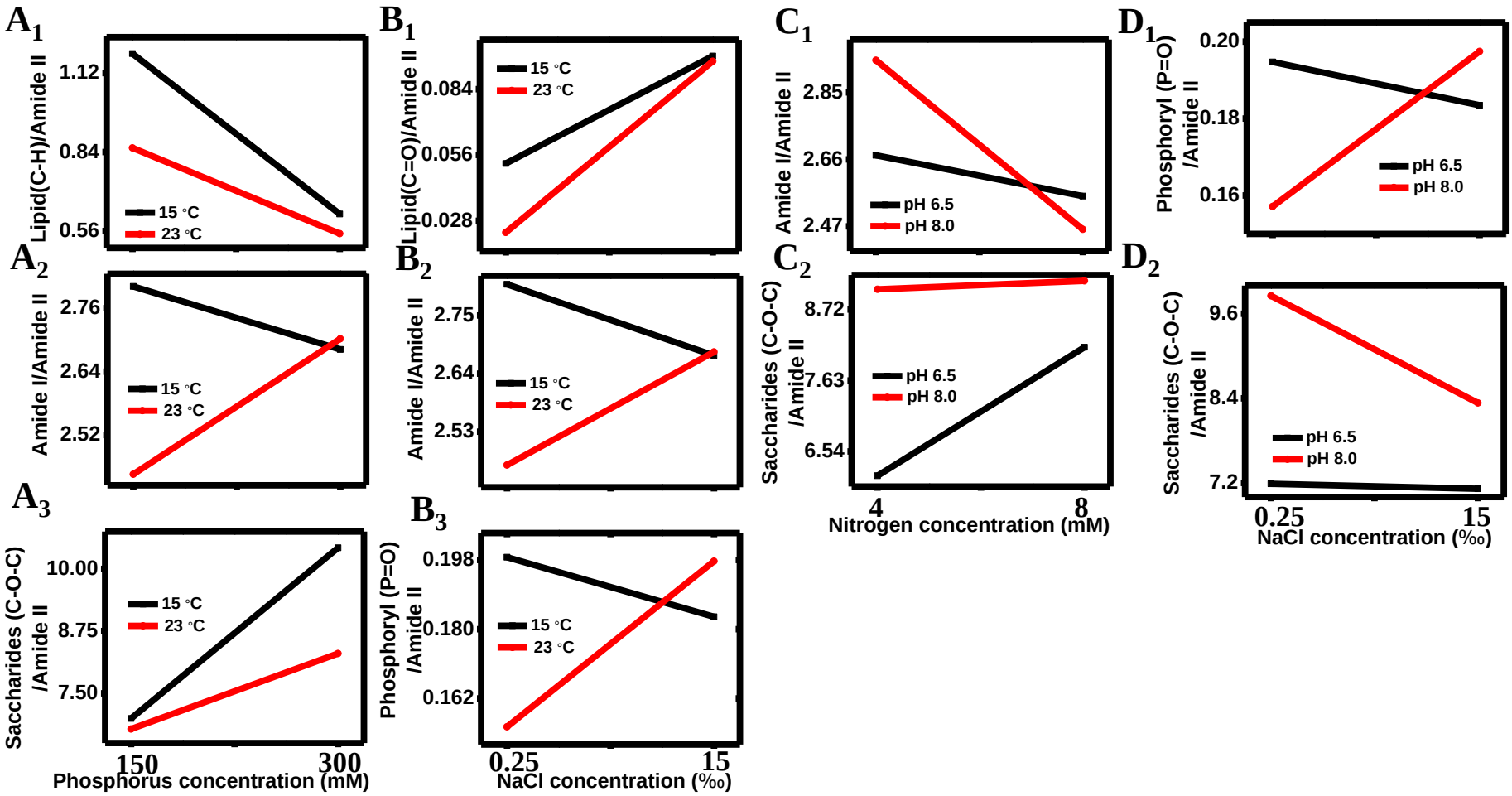


Figure S11. Remaining two-order interactions above the Bonferroni limit under triclosan exposure.

Table S1. Triclosan given with its molecular information, water solubility and application area.

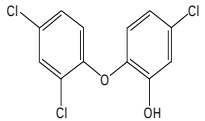
Characteristics	Triclosan
Molecular Formula	$C_{12}H_7Cl_3O_2$
Molecular Structure	
MW (g/mol)	289.54
Solubility (mg/L)	10
pKa	7.9
Application	Antimicrobial agent

Table S2. Independent variables of the 2^5 full factorial design.

Level of value	Temperature (℃)	pH	NaCl concentration (‰)	Nitrogen concentration (mM)	Phosphorus concentration (μM)
+1	23	8.0	15.00	8	300
-1	15	6.5	0.25	4	150

Table S3. Experimental Design matrix.

Std	Temperature (℃)		pH		NaCl concentration (‰)		Nitrogen concentration (mM)		Phosphorus concentration (μM)	
	Actual (x_1)	Coded (x_1)	Actual (x_2)	Coded (x_2)	Actual (x_3)	Coded (x_3)	Actual (x_4)	Coded (x_4)	Actual (x_5)	Coded (x_5)
1	15	-1	6.5	-1	0.25	-1	4	-1	150	-1
2	23	+1	6.5	-1	0.25	-1	4	-1	150	-1
3	15	-1	8.0	+1	0.25	-1	4	-1	150	-1
4	23	+1	8.0	+1	0.25	-1	4	-1	150	-1
5	15	-1	6.5	-1	15.00	+1	4	-1	150	-1
6	23	+1	6.5	-1	15.00	+1	4	-1	150	-1
7	15	-1	8.0	+1	15.00	+1	4	-1	150	-1
8	23	+1	8.0	+1	15.00	+1	4	-1	150	-1
9	15	-1	6.5	-1	0.25	-1	8	+1	150	-1
10	23	+1	6.5	-1	0.25	-1	8	+1	150	-1
11	15	-1	8.0	+1	0.25	-1	8	+1	150	-1
12	23	+1	8.0	+1	0.25	-1	8	+1	150	-1
13	15	-1	6.5	-1	15.00	+1	8	+1	150	-1
14	23	+1	6.5	-1	15.00	+1	8	+1	150	-1
15	15	-1	8.0	+1	15.00	+1	8	+1	150	-1
16	23	+1	8.0	+1	15.00	+1	8	+1	150	-1
17	15	-1	6.5	-1	0.25	-1	4	-1	300	+1
18	23	+1	6.5	-1	0.25	-1	4	-1	300	+1
19	15	-1	8.0	+1	0.25	-1	4	-1	300	+1
20	23	+1	8.0	+1	0.25	-1	4	-1	300	+1
21	15	-1	6.5	-1	15.00	+1	4	-1	300	+1
22	23	+1	6.5	-1	15.00	+1	4	-1	300	+1
23	15	-1	8.0	+1	15.00	+1	4	-1	300	+1
24	23	+1	8.0	+1	15.00	+1	4	-1	300	+1
25	15	-1	6.5	-1	0.25	-1	8	+1	300	+1
26	23	+1	6.5	-1	0.25	-1	8	+1	300	+1
27	15	-1	8.0	+1	0.25	-1	8	+1	300	+1
28	23	+1	8.0	+1	0.25	-1	8	+1	300	+1
29	15	-1	6.5	-1	15.00	+1	8	+1	300	+1
30	23	+1	6.5	-1	15.00	+1	8	+1	300	+1
31	15	-1	8.0	+1	15.00	+1	8	+1	300	+1
32	23	+1	8.0	+1	15.00	+1	8	+1	300	+1

Table S4. Significant factors of cell density and relative cellular components.

Cell Density	Lipid(C-H)/Amide II	Lipid (C=O)/Amide II	Amide I/Amide II	Phosphodiester (P=O)/Amide II	Saccharides (C-O-C)/Amide II
temperature * pH * NaCl concentration (-)	phosphorus (-)	NaCl concentration (+)	nitrogen (-)	nitrogen (-)	phosphorus (+)
nitrogen (-)	nitrogen * phosphorus (+)	temperature (-)	temperature * pH * nitrogen * phosphorus (+)	temperature * NaCl concentration (-)	nitrogen * phosphorus (-)
Temperature * pH * NaCl concentration * nitrogen (-)	nitrogen (-)	temperature * NaCl concentration (+)	temperature * pH * NaCl concentration (-)	NaCl concentration * nitrogen * phosphorus (-)	NaCl concentration * phosphorus (-)
NaCl concentration (-)	temperature (-)	phosphorus (-)	pH * nitrogen * phosphorus (-)	pH * NaCl concentration (-)	pH (+)
pH * NaCl concentration * nitrogen (+)	NaCl concentration (+)	NaCl concentration * nitrogen (-)	temperature * phosphorus (+)	nitrogen * phosphorus (+)	temperature (-)
NaCl concentration * nitrogen (+)	temperature * NaCl concentration * phosphorus (+)	pH (+)	pH * nitrogen (-)	pH * phosphorus (+)	nitrogen (+)
temperature * NaCl concentration * phosphorus (+)	temperature * pH (-)	temperature * nitrogen * phosphorus (-)	temperature * NaCl concentration (+)	temperature * pH * NaCl concentration * nitrogen * phosphorus (-)	temperature * phosphorus (-)

Temperature * nitrogen (+)	pH * NaCl concentration * phosphorus (+)	temperature * pH (-)	pH * NaCl concentration * phosphorus (+)	temperature (+)	pH * nitrogen (-)
	temperature * phosphorus (+)	temperature * NaCl concentration * phosphorus (+)	temperature * pH (-)	NaCl concentration (-)	pH * nitrogen * phosphorus (-)
	temperature * pH * NaCl concentration * nitrogen (+)	temperature * pH * NaCl concentration (-)	temperature (-)	temperature * phosphorus (-)	temperature * pH * phosphorus (-)
	temperature * pH * NaCl concentration * phosphorus (+)	temperature * NaCl concentration * nitrogen (-)	pH * phosphorus (+)		NaCl concentration (-)
	temperature * pH * NaCl concentration (-)	nitrogen (-)	NaCl concentration * nitrogen (+)		pH * NaCl concentration (-)
	temperature * nitrogen (+)	temperature * pH * nitrogen * phosphorus (+)			NaCl concentration * nitrogen * phosphorus (+)
	pH * NaCl concentration * nitrogen * phosphorus (+)	nitrogen * phosphorus (+)			pH * NaCl concentration * phosphorus (-)
	NaCl concentration * phosphorus (+)	pH * NaCl concentration * nitrogen (+)			pH * phosphorus (+)
	temperature * nitrogen * phosphorus (-)	temperature * nitrogen (+)			temperature * nitrogen (-)
	temperature * pH * phosphorus (+)				temperature * pH * NaCl concentration * nitrogen (-)

temperature * NaCl concentration * nitrogen * phosphorus (-)	temperature * pH * nitrogen * phosphorus (+)
	temperature * pH (-)
	temperature * nitrogen * phosphorus (-)
	temperature * NaCl concentration (+)
	temperature * NaCl concentration * nitrogen * phosphorus (+)

Note: The sequence is based on the contribution to the significant effects from the highest to the lowest. (+) stands for the positive effect; (-) stands for the negative effect.

Table S5. The contribution of all of main effects and interactions in toxicity assessment.

Term	Contribution (%)					
	Cell density	Lipid(C-H) /Amide II	Lipid (C=O) /Amide II	Amide I /Amide II	Phosphodiester (P=O)/Amide II	Saccharides (C-O-C)/Amide II
Temperature	2.438	5.635	6.574	3.933	2.826	4.040
pH	0.159	0.085	3.418	1.076	1.873	11.178
NaCl concentration	4.285	5.240	59.432	0.220	2.552	1.874
Nitrogen concentration	5.749	15.890	0.765	12.422	12.696	3.277
Phosphorus concentration	0.004	37.890	5.248	0.660	0.983	18.134
Temperature*pH	0.103	1.562	1.799	4.054	1.149	0.703
Temperature*NaCl concentration	0.143	0.069	5.475	4.264	8.862	0.595
Temperature*Nitrogen concentration	3.037	0.709	0.240	0.166	1.043	1.278
Temperature*Phosphorus concentration	0.541	1.344	0.027	4.937	2.462	2.706
pH*NaCl concentration	0.556	0.030	0.023	0.599	6.881	1.558
pH*Nitrogen concentration	0.426	0.004	0.026	4.635	0.002	2.523
pH*Phosphorus concentration	0.442	0.196	0.020	2.300	3.857	1.285
NaCl concentration*Nitrogen concentration	3.579	0.205	4.105	1.804	0.509	0.079
NaCl concentration*Phosphorus concentration	2.435	0.576	0.050	0.144	0.251	15.059
Nitrogen concentration*Phosphorus concentration	0.764	16.859	0.462	0.096	4.398	16.780
Temperature*pH*NaCl concentration	10.817	0.800	1.355	8.788	0.518	0.375
Temperature*pH*Nitrogen concentration	2.080	0.220	0.000	0.389	0.011	0.001
Temperature*pH*Phosphorus concentration	0.011	0.352	0.076	0.051	0.426	1.902
Temperature*NaCl concentration*Nitrogen concentration	0.169	0.059	0.934	0.000	1.526	0.000
Temperature*NaCl concentration*Phosphorus concentration	3.514	2.609	1.479	1.447	0.000	0.071

Temperature*Nitrogen concentration*Phosphorus concentration	1.211	0.379	3.409	0.088	0.012	0.641
pH*NaCl concentration*Nitrogen concentration	3.648	0.106	0.262	0.487	0.420	0.018
pH*NaCl concentration*Phosphorus concentration	0.186	1.516	0.000	4.114	0.670	1.298
pH*Nitrogen concentration*Phosphorus concentration	0.131	0.192	0.198	5.309	0.027	2.311
NaCl concentration*Nitrogen concentration*Phosphorus concentration	0.015	0.208	0.067	0.442	7.614	1.446
Temperature*pH*NaCl concentration*Nitrogen concentration	5.584	1.227	0.048	1.353	0.132	0.889
Temperature*pH*NaCl concentration*Phosphorus concentration	2.267	1.118	0.092	0.008	0.847	0.093
Temperature*pH*Nitrogen concentration*Phosphorus concentration	0.198	0.187	0.464	10.608	1.945	0.868
Temperature* NaCl concentration*Nitrogen concentration*Phosphorus concentration	0.229	0.239	0.092	0.115	0.013	0.526
pH*NaCl concentration*Nitrogen concentration*Phosphorus concentration	0.443	0.702	0.072	0.000	0.313	0.363
Temperature*pH*NaCl concentration*Nitrogen concentration*Phosphorus concentration	0.181	0.062	0.161	0.476	2.850	0.055

Table S6. Wavenumber (cm^{-1}) and assignment of the major bands in the synchrotron-based infrared spectra of *Chlorococcum* sp. cells.

Wavenumber (cm^{-1})	Vibrational mode assignment and main contribution
3000-2800	C-H stretch in acyl from fatty acids/lipids
~1740	C=O in ester and ester fatty acids.
1724-1588	C=O stretch of Amide I corresponding to protein secondary structure
1490-1585	N-H bend of Amide II of proteins
1200-1290	P=O from nucleic acids and phosphoryl group
953-1190	C-O-C mainly from polysaccharide rings

REFERENCES

(1) Coogan, M. A.; Edziyie, R. E.; La Point, T. W.; Venables, B. J. Algal bioaccumulation of triclocarban, triclosan, and methyl-triclosan in a North Texas wastewater treatment plant receiving stream. *Chemosphere* **2007,** *67*, (10), 1911-1918.

(2) Ding, T.; Lin, K.; Bao, L.; Yang, M.; Li, J.; Yang, B.; Gan, J. Biouptake, toxicity and biotransformation of triclosan in diatom Cymbella sp. and the influence of humic acid. *Environ. Pollut.* **2018,** *234*, 231-242.

(3) Xin, X.; Huang, G.; An, C.; Raina-Fulton, R.; Weger, H. Insights into Long-term Toxicity of Triclosan to Freshwater Green Algae in Lake Erie. *Environ. Sci. Technol.* **2019**, 53 (4), 2189-2198.

(4) Bothwell, M. L. Growth rate responses of lotic periphytic diatoms to experimental phosphorus enrichment: the influence of temperature and light. *Can. J. Fish. Aquat. Sci.* **1988,** *45*, (2), 261-270.

(5) Zhu, L.; Zhang, X.; Ji, L.; Song, X.; Kuang, C. Changes of lipid content and fatty acid composition of Schizochytrium limacinum in response to different temperatures and salinities. *Process Biochem.* **2007,** *42*, (2), 210-214.

(6) Chen, W.; Yang, H.; Chen, Y.; Xia, M.; Chen, X.; Chen, H. Transformation of nitrogen and evolution of N-containing species during algae pyrolysis. *Environ. Sci. Technol.* **2017**, 51 (11), 6570-6579.