Genotoxicity of disinfection by-products (DBPs) upon chlorination of nine different freshwater algal species at variable reaction time

Y. L. Zhang, B. P. Han, B. Yan, Q. M. Zhou and Y. Liang

ABSTRACT

Nine local dominant freshwater algal species, including green algae (*Chlorella* sp., *Chlamydomonas* sp. and *Scenedesmus quadricauda*), diatom (*Navicula pelliculosa*, *Nitzschia palea Grunow* and *Synedra* sp.), blue-green algae (*Microcystis* sp., *Chroococcus* sp. and *Gloeocapsa* sp.) were isolated from source water reservoirs and cultivated in the laboratory. The algal biomass was chlorinated (20 °C, pH 7, residual chlorine 2 mg L^{-1}). Yields of chloroform, dichloroacetic acid, trichloroacetic acid, dichloroacetonitrile and trichloroacetonitrile and genotoxicity of the chlorinated solutions at eight chlorination intervals (0.5, 1, 2, 5, 10, 30, 60 and 120 min) were determined via SOS-ChromoTest and Comet assay. The results showed that green algae and diatom were more effective disinfection by-products (DBPs) precursors than blue-green algae. Genotoxicity was shown to be chlorination time-dependent, in agreement with our previous findings, suggesting that intermediate DBPs rather than trihalomethanes or haloacetic acids were major contributors to the genotoxicity of chlorinated solutions.

Key words | algal species, Caco-2 cells, chlorination time, Comet assay, genotoxicity, SOS-ChromoTest

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INTRODUCTION

Chlorination is widely used in water disinfection due to its low cost and effectiveness in eliminating pathogenic microorganisms. Disinfection by-products (DBPs) are generated when free chlorine reacts with natural organic matter in the source water. There is considerable epidemiological evidence for urinary bladder cancer being causally associated with chlorination DBPs, and that evidence for colon and rectal cancer is suggestive (IARC 2004). Currently nearly 700 DBPs have been reported in the literature, but none of the identified chlorination DBPs seems to be a plausible bladder carcinogen (Hrudey 2009). Emerging DBPs such as haloquinones, halo-cyclopentene and cyclohexene derivatives are likely to be of health concern, according to a recent analysis based on quantitative structure toxicity relationship (Bull *et al.* 2011). These DBPs appear to be intermediate DBPs during disinfection and can be further oxidized (e.g. with prolonged chlorination), while intermediate DBPs are likely to be of health concern since the

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mutagenicity of the chlorinated algal fractions (cells and proteins) reached a peak level shortly after chlorination and then declined afterwards, different from that of chloroform ($CHCl_3$) generation (Lui *et al.* 2011, 2012).

In contrast with many other places, where as much as 50% of dissolved organic carbon (DOC) is composed mainly of humic substances in most natural waters (Nikolaou *et al.* 2001), in the Pearl River Delta region (China), the DBP precursors in some drinking water reservoirs are primarily from algae (Hong *et al.* 2008a). During the algal blooming seasons, the increase in algal biomass and algal-derived organic matter may contribute to total organic carbon (TOC) and DBP formation. Algae are the major sources of dissolved organic nitrogen (DON) in natural waters (Westerhoff & Mash 2002). Algae cells contain a wide range of organic nitrogen compounds, such as polysaccharides, proteins, peptides, etc. These organic compounds may contribute to DBPs formation and particularly to prominent DBP species such as trihalomethanes (THMs) and haloacetic acids (HAAs) (Scully *et al.* 1988; Hureiki *et al.* 1994; Westerhoff & Mash 2002). DON can be transformed to nitrogen-containing DBPs (N-DBPs) during chlorination or chloramination and non-regulated N-DBPs are considered more toxic than the regulated THMs and HAAs (Richardson *et al.* 2007).

The role of algae in DBP formation has been investigated in several studies (Wardlaw *et al.* 1991; Graham *et al.* 1998; Glezer *et al.* 1999; Plummer & Edzwald 2001; Nguyen *et al.* 2005; Hong *et al.* 2008a; Lui *et al.* 2011, 2012). Both the nature of precursors and treatment conditions (e.g. pH, reaction time, temperature and relative amount of chlorine) determine concentration and composition of DBPs (Amy *et al.* 1998), which in turn result in different toxicities in chlorinated drinking water (Marabini *et al.* 2006; Pereira *et al.* 2007; Shi *et al.* 2009). Composition of algal cells plays an important role in DBPs formation during chlorination. Generally, blue-green algae have a higher protein content than diatoms, while diatoms accumulate more lipids than blue-green algae and green algae (Hong *et al.* 2008a). For algal cells, extracellular organic matter and algal fractions (cells and proteins) of different algae species were also reported to produce DBPs in different compositions (Wardlaw *et al.* 1991; Huang *et al.* 2009; Lui *et al.* 2011). The majority of the DBP precursors (70%) was attributable to the cellular material (Plummer & Edzwald 2001).

In this study, nine dominant local freshwater algal species, including green algae (chlorophyta), blue-green algae (cyanobacteria) and diatoms (bacillariophyta), were isolated in the source water (Hu *et al.* 2003; Liang *et al.* 2008). It was reported that the DOC of surface water is about 1.69–2.18 mg L^{-1} in this region (Liang *et al.* 2008). We use a DOC concentration of 50 mg L^{-1} to quantify algal cells to standardize organic precursor concentration among nine algal species, so that chlorine consumption and DBPs production could be easily compared. Changes in genotoxicity at different chlorination times were examined using *Escherichia coli* SOS-ChromoTest and Comet assay using human Caco-2 cells. The major objectives were: (1) to identify algal species as effective DBPs precursors in the source water; and (2) to identify a pattern of genotoxicity as a function of chlorination time. We hope information from this study could contribute to controlling DBPs precursors in the source water and formation of genotoxic DBPs in chlorination.

MATERIALS AND METHODS

Algal culture

Nine species of algae (chlorophyta: *Chlorella* sp., *Chlamydomonas* sp. and *Scenedesmus quadricauda*; cyanophyta: *Microcystis* sp., *Chroococcus* sp. and *Gloeocapsa* sp.; diatom: *Navicula pelliculosa*, *Nitzschia palea Grunow* and *Synedra* sp.) algae were obtained from the Limnology Institute of Jinan University, previously isolated in reservoirs at the Pearl River Delta region. Blue-green and the green alga were cultured in BG11 medium (Stainer *et al.* 1971) and diatoms in diatom medium (DM) in 5 L glass jars, at 25 ± 2 °C under 1000 lux of illumination with a 14:10 h light:dark cycle. Daily counting was undertaken using a light microscope and optical density (OD 680 nm) was used to quantify the algal biomass. When the algal growth reached log phase, the algal biomass was harvested by centrifugation (3000 rpm, 10 min) and rinsed using Milli-Q water. The pellet was regarded as the algal cell fraction and stored at −80 °C, avoiding light.

Chlorination

The algal cell pellets were crushed using a liquid nitrogen grinding method and solutions containing algal-derived

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organic materials (with starting DOC of 50 mg C L^{-1}) were used for chlorination. Chlorination was conducted with eight different chlorine contact times (0.5, 1, 2, 5, 10, 30, 60 and 120 min). All samples were dissolved in phosphate buffer (pH 7, 0.2 M NaH_2PO_4 and 0.2 M NaOH) to maintain the pH. DOC was detected using a TOC analyzer (Shimadzu TOC 5000). The stock chlorine solution (NaOCl, 49.7 mg Cl_2 mL^{-1}) was standardized by the *N,N*-diethyl-*p*-phenylenediamine titrimetric method (APHA 1998). Excess chlorine was applied based on a chlorine demand test conducted beforehand to ensure a substantial residual of chlorine (about 2 mg L^{-1}) after the chlorination period. All chlorination experiments were conducted at 20 °C in 80 mL glass tubes with glass septa in the dark. At the end of the chlorination period, samples for THM analyses were collected, head-space free, in 20 mL glass vials containing $Na_2S_2O_3$ quenching agent, while samples for HAA analyses were collected in vials with NH_4Cl quenching agent. Residue chlorine of samples for the toxicity test was quenched with a moderate amount of Na_2SO_3.

DBPs analysis

DBPs including $CHCl_3$, dichloroacetic acid (DCAA), trichloroacetic acid (TCAA), dichloroacetonitrile (DCAN) and trichloroacetonitrile (TCAN) were analyzed. Briefly, $CHCl_3$ was extracted by pentane and determined according to the Standard Method (APHA 1998). DCAA and TCAA were extracted by methyl tert-butyl ether (MTBE) and methylated by acidic methanol, and determined following USEPA (2003). DCAN and TCAN were extracted by MTBE, and analyzed according to the USEPA (1995). 1,2-Dibromopropane was used as the internal standard. The DBPs were determined by a GC-ECD system (with a 30 m × 0.25 mm × 0.25 μm HP-5 capillary column) using nitrogen as the carrier gas. The recovery rates for $CHCl_3$, DCAA, TCAA, DCAN, TCAN, 1,1-DCP and 1,1,1-TCP were 97.5 ± 6.34, 101 ± 8.4, 97.3 ± 5.3, 90.7 ± 4.7, 87.7 ± 10.4, 102.7 ± 10.4 and 105.6 ± 8.2, respectively. The detection limits for $CHCl_3$, DCAA and TCAA were 0.00938, 0.0105 and 0.00894 μmol L^{-1}, respectively.

SOS-ChromoTest

SOS-ChromoTest was initially performed without metabolic activation using an SOS-ChromoTest Kit and *E. coli* PQ37 strain supplied by EBPI (Brampton, Ontario, Canada), which was then modified according to Quillardet & Hofnung (1985) and Mersch-Sundermann *et al.* (1991). Briefly, the bacteria were first incubated in growth medium for 8–12 hours at 37 °C. The bacterial suspension was diluted using 10% DMSO in sterile 0.85% saline to OD 0.05–0.06 at 600 nm. Six concentration levels of positive controls (4 Nitro Quinoline Oxide, 4NQO) and chlorinated algal solutions were prepared respectively by two-fold dilution using 10% DMSO saline. After adding 10 μL samples into microwells, 100 μL of bacteria suspension were transferred into each well. The microplate was incubated at 37 °C for 2 h. Activities of *β*-galactosidase and alkaline phosphatase were then determined using 5-bromo-4-chloro-3-indolyl-*β*-D-galactoside (X-gal, MYM Biological Technology Co. Ltd) and *p*-nitrophenyl phosphate (PNPP, MYM Biological Technology Co. Ltd) disodium substrates, respectively. The ratio of the activities, *β*-galactosidase/alkaline phosphatase, divided by the values of control samples (10% DMSO) was calculated as the induction factor. The induction factor increased with sample dose, and SOS inducing potency (SOSIP) was calculated as the slope of the linear region.

Comet assay using Caco-2 cells

Human Caco-2 cell line was obtained from the American Type Culture Collection (ATCC, Rockville, MD). The cells were cultured in Dulbecco Modified Eagle's Medium (DMEM) supplemented with 20% fetal bovine serum (FBS), in a humidified atmosphere of 95% air and 5% CO_2 at 37 °C. The medium was changed every 3 days and the cells were sub-cultured every 7 days when 95% cells confluenced.

Oxiselect Comet Assay Kit (Cell Biolabs, Inc.) was used for Comet assays. Procedures mainly followed the product manual with some modifications according to Fairbairn *et al.* (1995) and Klaude *et al.* (1996). Briefly, Caco-2 cells were incubated in a 12-well plate at a density of 1×10^5 cells per well in 1 mL DMEM with 20% FBS. After 24 h, cells settled and formed a monolayer. Cell viability was determined via observing the color change of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). Only cells with viability of more than 80% were used for toxicity tests. The cells were then washed

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with phosphate-buffered saline (PBS, without Mg^{2+} and Ca^{2+}) and treated with different samples, and cells treated simply with DMEM medium (Milli-Q water prepared) were used as the control. The MEM powder was dissolved directly into the chlorinated solutions, followed by filtration through 0.22 μm acetate cellulose membrane before incubation with the cells. After treatment for 1 h (at 37 °C, with 5% CO_2), the cells were collected and washed once with ice-cold PBS, centrifuged, the supernatant discarded and suspended in 100 μL ice-cold PBS. The cells then were mixed with low melting point agarose (37 °C) 1:10 briefly and spread on a slide using 75 μL mixture per well, at 4 °C, avoiding light, until solidification. The cells were lysed in lysis buffer at 4 °C for 1 h, and then in alkaline buffer for 30 min (protected from light), followed by washing with ice-cold Tris-borate-EDTA (TBE) buffer twice. Electrophoresis was

Table 1 | $CHCl_3$, DCAN and TCAN formation (mean ± SD, μmol L^{-1}) among nine species during different chlorination time

	Chlorophyta			Diatom			Cyanophyta		
Time (min)	CHLO	CHLA	SCEN	NAVI	NITZ	SYNE	MICR	CHRO	GLOE
$CHCl_3$									
0.5	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
1	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
2	N.D.	N.D.	17.9 ± 0.106[a]	16.6 ± 2.01[ab]	N.D.	9.49 ± 2.54[c]	N.D.	9.74 ± 2.34[c]	N.D.
5	N.D.	31.0 ± 0.991[a]	36.8 ± 0.0277[a]	6.25 ± 0. 103[e]	11.4 ± 3.96[cde]	13.8 ± 0.656[bc]	N.D.	18.4 ± 0.214[b]	9.26 ± 0.182[de]
10	8.56 ± 0.111[e]	36.1 ± 0.053[ab]	29.5 ± 0.240[c]	10.9 ± 1.42[e]	32.7 ± 2.62[bc]	16.7 ± 0.336[d]	10.1 ± 0.278[e]	38.7 ± 1.74[a]	16.1 ± 0.101[d]
30	25.3 ± 0.293[abc]	61.8 ± 0.247[ab]	63.7 ± 0.100[a]	22.8 ± 0.158[abc]	44.9 ± 8.45[abc]	27.8 ± 0.207[abc]	9.99 ± 0.100[c]	41.2 ± 29.2[bc]	23.5 ± 7.82[abc]
60	54.3 ± 15.2[c]	99.2 ± 1.12[ab]	108 ± 0.111[a]	15.3 ± 6.37[c]	88.3 ± 6.48[ab]	38.8 ± 1.48[c]	17.5 ± 0.378[c]	45.8 ± 0.00[c]	30.1 ± 1.64[c]
120	136 ± 4.14[b]	136 ± 1.18[b]	168 ± 0.578[a]	43.3 ± 0.386[f]	106 ± 5.12[c]	54.9 ± 11.4[d]	29.2 ± 0.272[g]	77.8 ± 0.0404[e]	31.3 ± 3.18[g]
DCAN									
0.5	49,197 ± 244[a]	24.8 ± 6.34[bc]	270 ± 72.9[bc]	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
1	53.2 ± 0.788[c]	29.2 ± 3.90[c]	472 ± 51.0[a]	N.D.	14.0 ± 0.962[c]	N.D.	199 ± 27.2[b]	10.3 ± 6.23[c]	N.D.
2	4,330 ± 205[a]	28.0 ± 6.87[b]	52.7 ± 26.4[b]	13.4 ± 0.236[b]	10.6 ± 2.27[b]	48.0 ± 5.56[b]	111 ± 94.0[b]	9.64 ± 2.92[c]	N.D.
5	86.2 ± 3.14[ab]	188 ± 161[ab]	130 ± 46.3[ab]	34.4 ± 10.0[ab]	N.D.	9.24 ± 0.552[b]	272 ± 33.0[a]	11.4 ± 0.489[b]	N.D.
10	78.6 ± 0.126[b]	155 ± 7.63[a]	23.7 ± 6.43[c]	49.0 ± 7.72[bc]	N.D.	N.D.	78.4 ± 19.5[b]	15.5 ± 0.883[c]	N.D.
30	95.8 ± 4.45[b]	63.4 ± 9.65[bc]	247 ± 12.6[a]	21.4 ± 2.21[d]	43.0 ± 16.3[cd]	14.8 ± 11.7[d]	18.4 ± 5.28[d]	13.2 ± 1.58[d]	19.0 ± 3.07[d]
60	15.5 ± 2.76[c]	1,730 ± 71.4[a]	729 ± 469[b]	24.8 ± 0.835[c]	9.25 ± 0.788[c]	19.9 ± 1.56[c]	31.4 ± 14.3[c]	12.8 ± 5.12[c]	15.3 ± 3.94[c]
120	17.7 ± 1.21[c]	21.0 ± 3.39[c]	40.3 ± 4.18[b]	60.2 ± 1.33[a]	N.D.	N.D.	35.1 ± 1.50[b]	N.D.	N.D.
TCAN									
0.5	2,690 ± 152[a]	28.9 ± 0.693[b]	68.1 ± 19.7[b]	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
1	32.1 ± 4.51[b]	51.5 ± 1.24[b]	84.0 ± 12.0[b]	N.D.	94.2 ± 19.7[b]	N.D.	963 ± 233a	N.D.	N.D.
2	2,380 ± 113[b]	140 ± 6.96[d]	3,260 ± 29.7[a]	N.D.	797 ± 40.1[c]	75.3 ± 21.0[d]	17.6 ± 4.31[d]	N.D.	N.D.
5	47.4 ± 1.73[e]	300 ± 0.910[b]	137 ± 2.84[c]	N.D.	465 ± 0.780[a]	11.5 ± 0.633[f]	128 ± 9.34[cd]	N.D.	109 ± 13.4[d]
10	43.2 ± 0.0693[c]	215 ± 2.64[b]	35.4 ± 1.26[c]	N.D.	223 ± 37.7[b]	N.D.	50.0 ± 2.31[c]	542 ± 29.9[a]	N.D.
30	50.1 ± 1.18[cd]	173 ± 37.0[b]	17.2 ± 0.568[d]	9.72 ± 2.81[d]	1,440 ± 23.9[a]	36.2 ± 0.711[cd]	36.8 ± 8.54[cd]	230 ± 8.10[b]	92.5 ± 27.6[c]
60	N.D.	88.4 ± 4.36[bc]	323 ± 63.8[a]	12.7 ± 4.89[c]	168 ± 19.0[b]	20.0 ± 0.793[c]	45.7 ± 14.7[c]	176 ± 22.2[b]	10.6 ± 1.43[c]
120	9.75 ± 0.667[d]	55.5 ± 2.11[ab]	N.D.	22.5 ± 4.42[d]	77.8 ± 2.46[a]	N.D.	46.0 ± 5.42[bc]	24.1 ± 12.2[cd]	N.D.

Means containing the same letter were not significantly different ($p > 0.05$), according to multiple-comparison of means by one-way ANOVA. Detection limits of $CHCl_3$, DCAN and TCAN were 9.38, 9.00 and 9.42 pmol L^{-1} respectively. N.D.: not detectable; CHLO: *Chlorella* sp.; CHLA: *Chlamydomonas* sp.; SCEN: *Scenedesmus quadricauda*; NAVI: *Navicula pelliculosa*; NITZ: *Nitzschia palea Grunow*; SYNE: *Synedra* sp.; MICR: *Microcystis* sp.; CHRO: *Chroococcus* sp.; GLOE: *Gloeocapsa* sp.

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performed at 4 °C, 1 V cm^{-1}, for 15 min in TBE. Afterwards, the slides were neutralized using pre-chilled DI water, dehydrated with 70% ethanol, and stained using vista green DNA dye for 15 min. The cells were measured for their comet tail moment (TM) using a fluorescence microscope with a CCD camera, to indicate DNA damage in the treated cells. About 50 cells per treatment were randomly measured, and the same level of treatment was conducted in three different generations of cells. Values of average TM were calculated from 50 cells exposed to the same level of treatment.

Data analysis

One-way analysis of variance (ANOVA) and independent *t*-test were used to compare the means analyzed using the SPSS (Statistic Package for Social Science) software program for Microsoft Windows (Version 16.0) (SPSS Inc.).

RESULTS AND DISCUSSION

DBPs yields among different algal species

In general, algal organic matter upon chlorination was more effective in generating $CHCl_3$ than the other measured DBPs (Tables 1 and 2). In particular, among different species, green algae were shown to form more $CHCl_3$ upon chlorination than the other algal species. At 120 min chlorination time, the highest yield of $CHCl_3$ was observed to be formed from *Scenedesmus quadricauda* (168 μmol L^{-1}), 5.75 times of that from *Microcystis* sp.

Table 2 | DCAA and TCAA formation (mean ± SD, μmol L^{-1}) among nine species during different chlorination time

	Chlorophyta			Diatom			Cyanophyta		
Time (min)	CHLO	CHLA	SCEN	NAVI	NITZ	SYNE	MICR	CHRO	GLOE
DCAA									
0.5	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
1	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	60.2 ± 16.0	N.D.	N.D.
2	13.6 ± 3.61[a]	N.D.	N.D.	13.8 ± 5.12[a]	19.5 ± 0.413[a]	15.3 ± 1.36[a]	N.D.	N.D.	11.6 ± 0.867[a]
5	12.5 ± 1.18[c]	N.D.	N.D.	46.2 ± 4.07[b]	N.D.	87.0 ± 13.2[a]	21.6 ± 3.41[c]	28.5 ± 0.335[bc]	35.6 ± 0.956[bc]
10	113 ± 1.40[ab]	N.D.	17.2 ± 2.39[c]	31.7 ± 7.55[c]	32.0 ± 4.39[c]	N.D.	N.D.	133 ± 11.4[a]	84.2 ± 1.24[b]
30	18.5 ± 0.447[b]	N.D.	33.6 ± 1.45[b]	N.D.	105 ± 9.57[a]	22.1 ± 2.27[b]	N.D.	N.D.	35.3 ± 0.0576[b]
60	19.4 ± 0.440[a]	N.D.	N.D.	16.4 ± 0.972[a]	16.4 ± 3.58[a]	14.3 ± 1.20[a]	19.0 ± 0.369[a]	31.1 ± 11.1[a]	26.1 ± 1.47[a]
120	26.2 ± 2.06[bc]	N.D.	12.5 ± 3.29[c]	18.9 ± 5.53[bc]	18.3 ± 3.41[bc]	N.D.	41.9 ± 3.82[b]	182 ± 12.5[a]	N.D.
TCAA									
0.5	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
1	10.8 ± 0.246	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
2	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	12.4 ± 3.80
5	361 ± 50.1[a]	N.D.	N.D.	13.9 ± 2.93[b]	N.D.	N.D.	10.4 ± 1.22[b]	46.2 ± 8.55[b]	N.D.
10	N.D.	N.D.	448 ± 84.2[a]	N.D.	N.D.	N.D.	9.06 ± 0.538[b]	16.7 ± 0.661[b]	N.D.
30	N.D.	N.D.	19.3 ± 4.41[a]	N.D.	12.9 ± 2.75[a]	N.D.	N.D.	N.D.	N.D.
60	N.D.	N.D.	N.D.	11.4 ± 4.04[a]	N.D.	N.D.	N.D.	N.D.	11.1 ± 1.46[a]
120	N.D.	N.D.	18.8 ± 0.369	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

Means containing the same letter were not significantly different ($p > 0.05$), according to multiple-comparison of means by one-way ANOVA. Detection limits of DCAA and TCAA were 10.5 and 8.94 pmol L^{-1} respectively. N.D.: not detectable; CHLO: *Chlorella* sp.; CHLA: *Chlamydomonas* sp.; SCEN: *Scenedesmus quadricauda*; NAVI: *Navicula pelliculosa*; NITZ: *Nitzschia palea Grunow*; SYNE: *Synedra* sp.; MICR: *Microcystis* sp.; CHRO: *Chroococcus* sp.; GLOE: *Gloeocapsa* sp.

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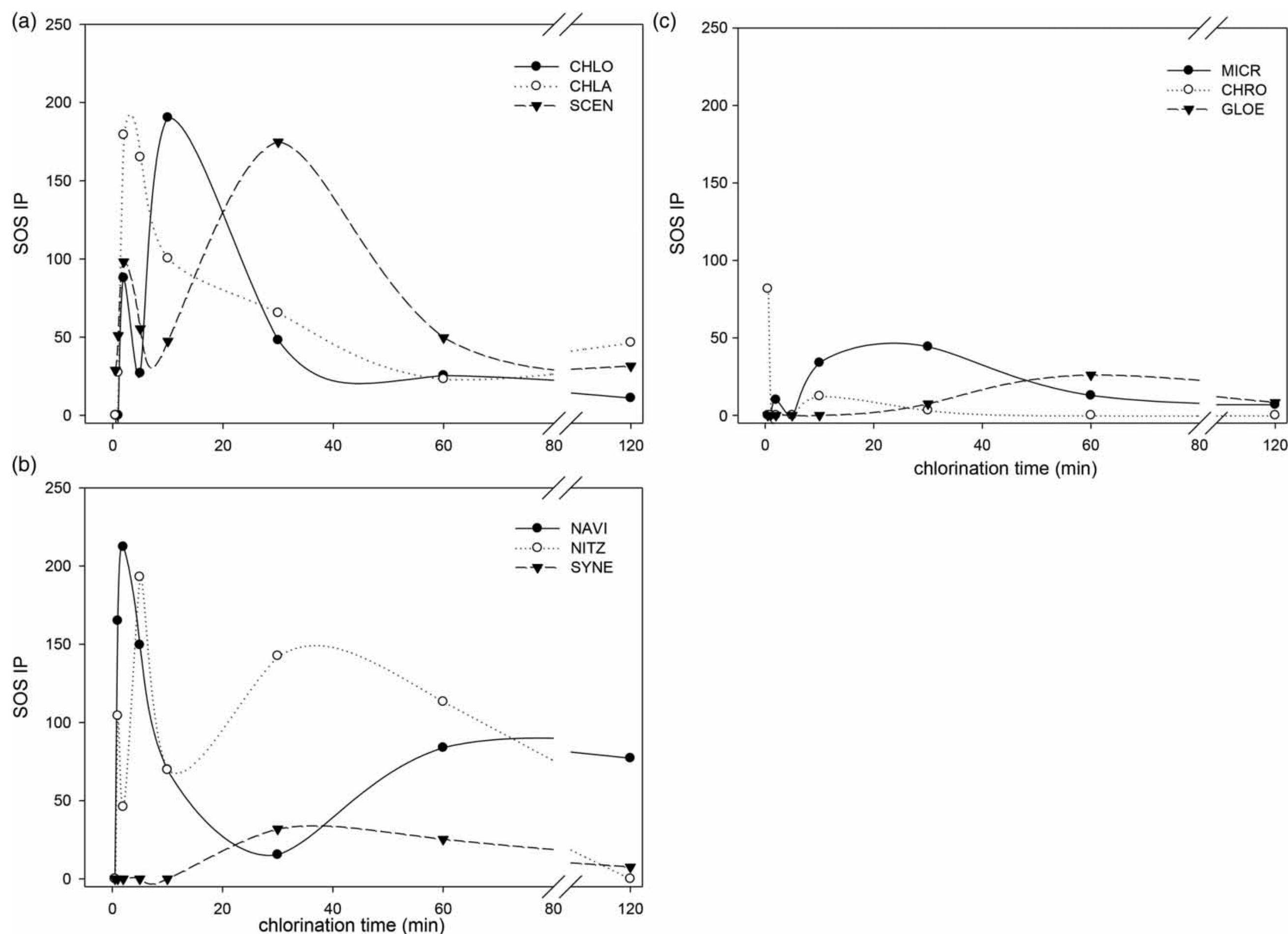

Figure 1 | Time-dependent changes in genotoxicity of chlorinated algae using SOS-test: (a) SOSIP change among green algae; (b) SOSIP among diatom; (c) SOSIP among blue-green algae. CHLO: *Chlorella* sp.; CHLA: *Chlamydomonas* sp.; SCEN: *Scenedesmus quadricauda*; NAVI: *Navicula pelliculosa*; NITZ: *Nitzschia palea Grunow*; SYNE: *Synedra* sp.; MICR: *Microcystis* sp.; CHRO: *Chroococcus* sp.; GLOE: *Gloeocapsa* sp.

(29.2 μmol L^{-1}). Meanwhile, it is also observed that different algal species in the same group had significant differences in $CHCl_3$ formation. For example, at 120 min, *Nitzschia palea Grunow* (diatom) produced relatively more $CHCl_3$ (106 μmol L^{-1}) than the other two diatoms (*Navicula pelliculosa* 43.3 μmol L^{-1} and *Synedra* sp. 54.9 μmol L^{-1}); *Chroococcus* sp. (blue-green algae) produced 77.8 μmol L^{-1} $CHCl_3$, twice higher than the other two blue-green algal species (*Microcystis* sp. 29.2 μmol L^{-1} and *Gloeocapsa* sp. 31.3 μmol L^{-1}). The comparison among different algal species was similar in DCAN and TCAN formation (Table 1), as these two DBPs are generally regarded as intermediate chlorination products in the process of $CHCl_3$ formation (Peters *et al.* 1990; Nikolaou *et al.* 2000; Reckhow *et al.* 2001). Particularly, DCAN formation was the highest (0.492 μmol L^{-1}) at 0.5 min chlorination of *Chlorella sp*., and the highest TCAN (3.26 μmol L^{-1}) was observed at 2 min chlorination of *Scenedesmus quadricauda*. The data were consistent with a previous study by Nguyen *et al.* (2005) that green algae were more productive in generating $CHCl_3$ than blue-green algae and diatoms (Nguyen *et al.* 2005). However, in our previous report of $CHCl_3$ formation after 4 d chlorination, the diatom was shown to be the most effective precursor (Hong *et al.* 2008b). Obviously chlorination time is another key factor in determining $CHCl_3$ generation. On the other hand, *Chroococcus* sp. (blue-green algae) formed the highest concentration of DCAA (peak level for 0.182 μmol L^{-1} at 120 min chlorination)

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among all the algal species, while minor amounts of TCAA were generated (Table 2). Whether this is due to generally higher protein in blue-green algae (Hong *et al.* 2008b) requires investigation in the future.

Genotoxicity

A preliminary study was conducted using unchlorinated algal cells at 50 mg L^{-1} TOC, and no genotoxicity was detected. All chlorinated solutions showed genotoxicity (Figure 1), and chlorination time appeared to be a critical contributor. Most solutions showed that peak levels of genotoxicity were reached before 30 min (except for *Gloeocapsa* sp. at 60 min) and then reduced when chlorination time was prolonged. A similar pattern was observed previously (Lui *et al.* 2011, 2012), suggesting that intermediate by-products generated within a very short reaction time were responsible for the fluctuation. A general pattern is observed that chlorinated green algal solutions, containing higher levels of $CHCl_3$, DCAN and TCAN (Table 1 and Figures 1 and 2), were more genotoxic. Both chlorinated *Synedra* sp. (diatom) and *Gloeocapsa* sp. (blue-green algae) formed relatively less DBPs (especially for DCAN and TCAN) during chlorination (Table 1) and also showed relatively lower genotoxicity (Figure 1). However, the link between the genotoxicity and any of the DBP level was not significant and therefore the specific responsible DBPs were not identified. Chlorination time is determined by raw water quality in the water treatment, particularly microbiological quality, while short chlorination time does not always mean less DBPs formation and the associated genotoxicity. Therefore, a sound control of chlorination time means a good reduction in both pathogens and genotoxic DBPs in water treatment.

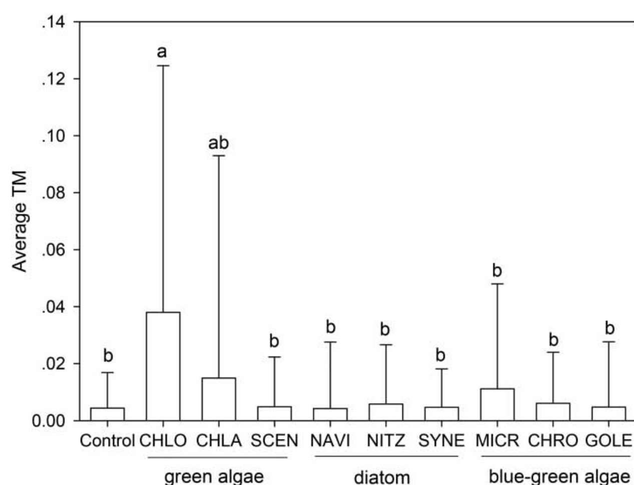

Figure 2 | Average tail moment of Caco-2 cells by the Comet assay, comparing among different algal species. Means containing the same letter were not significantly different ($p > 0.05$), according to multiple-comparison of means by one-way ANOVA. CHLO: *Chlorella* sp.; CHLA: *Chlamydomonas* sp.; SCEN: *Scenedesmus quadricauda*; NAVI: *Navicula pelliculosa*; NITZ: *Nitzschia palea Grunow*; SYNE: *Synedra* sp.; MICR: *Microcystis* sp.; CHRO: *Chroococcus* sp.; GLOE: *Gloeocapsa* sp.

CONCLUSIONS

DBPs formation was different among nine different species of algal cells during chlorination. $CHCl_3$ formation increased as chlorination time increased while the other DBPs (DCAA, TCAA, DCAN and TCAN) showed a peak level before 120 min. Green algae generally formed a higher level of $CHCl_3$. DCAN and TCAN also reached higher levels within 120 min. Blue-green algae produced relatively lower DBPs and also showed lower genotoxicity than green algae and diatom. DBPs formation and toxicity differs among different algal groups, and also different algal species in the same group.

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