

Toxicity of atrazine and its bioaccumulation and biodegradation in a green microalga, *Chlamydomonas mexicana*

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Received: 9 February 2014 / Accepted: 4 June 2014 / Published online: 15 June 2014
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Abstract This study evaluated the toxicity of herbicide atrazine, along with its bioaccumulation and biodegradation in the green microalga *Chlamydomonas mexicana*. At low concentration ($10 \mu\text{g L}^{-1}$), atrazine had no profound effect on the microalga, while higher concentrations (25, 50, and $100 \mu\text{g L}^{-1}$) imposed toxicity, leading to inhibition of cell growth and chlorophyll *a* accumulation by 22 %, 33 %, and 36 %, and 13 %, 24 %, and 27 %, respectively. Atrazine 96-h EC₅₀ for *C. mexicana* was estimated to be $33 \mu\text{g L}^{-1}$. Microalga showed a capability to accumulate atrazine in the cell and to biodegrade the cell-accumulated atrazine resulting in 14–36 % atrazine degradation at 10 – $100 \mu\text{g L}^{-1}$. Increasing atrazine concentration decreased the total fatty acids (from 102 to 75mg g^{-1}) and increased the unsaturated fatty acid content in the microalga. Carbohydrate content increased gradually with the increase in atrazine concentration up to 15 %. This study shows that *C. mexicana* has the capability to degrade atrazine and can be employed for the remediation of atrazine-contaminated streams.

Keywords Atrazine · *Chlamydomonas mexicana* · Bioaccumulation · Biodegradation · Fatty acid · Carbohydrate

Introduction

Herbicides have emerged as an indispensable part of modern agricultural practice, and their unregulated and indiscriminate application has raised concerns, as they have deleterious effects on diverse ecosystems in the vicinity of contaminated sites. Atrazine is used to efficiently control broadleaf and grassy weeds in agriculture and is one of the most widely used herbicides in the world (Vonberg et al. 2013). Atrazine interacts with the proteins of photosystem II at the plastoquinone B binding site and blocks the photosynthetic electron transport (Rutherford and Krieger-Liszkay 2001), thereby preventing CO₂ fixation and subsequent energy production in target weeds. Death of the target weeds occurs mainly due to desiccation following membrane damage, because of formation of reactive oxygen species that trigger a chain reaction of lipid peroxidation (Marchetti et al. 2013). Its widespread application, persistence, and mobility have led to its frequent detection in ground and surface water sources (Hayes et al. 2010) at concentrations exceeding $10 \mu\text{g L}^{-1}$ (USEPA 2012). Atrazine-induced detrimental effects on the aquatic ecosystem and alterations in aquatic community structure have been reported previously (Graymore et al. 2001; Weiner et al. 2004). Atrazine has also been reported to affect the human endocrine, central nervous, immune, and reproductive systems; therefore, an efficient removal strategy needs to be developed (Liu et al. 2014).

Various techniques such as adsorption by activated carbon, chemical extraction (Schroder et al. 2002), advanced oxidation process (Lieu et al. 2000), ultrasonic destruction (Collings and Gwan 2010), nanofiltration (Bodalo et al. 2010), zero valent iron (Dombek et al.

Responsible editor: Thomas Braunbeck

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2001), and photocatalytic degradation (Lackhoff and Reinhard 2002) have been reported for atrazine removal, but these methods have disadvantages that include low efficiency, high cost, and secondary pollution due to sludge generation. Atrazine degradation using bacteria (Kolekar et al. 2014) and fungi (Nwachukwu and Osuji 2007) has also been investigated. However, the high energy costs and supplementary nutritional requirements make these methods less feasible for implementation in the field as an economic remediation strategy (Subashchandrabose et al. 2013). Microalgae can serve as a potential sink for the removal of pesticides due to their endogenous catabolic systems, heterotrophic capability, and role in the fixation and turnover of carbon (Semple et al. 1999). Microalgal degradation of different organic xenobiotic compounds such as phenolic compounds (Pinto et al. 2002), pesticides (Bi et al. 2012; Zhang et al. 2011), endocrine-disrupting chemicals (Hirooka et al. 2005), and polyaromatic compounds (Mostafa et al. 2011) has been reported. Bioremediation using microalgae also provides additional benefits such as flexibility to handle fluctuations in the quality and quantity of contaminated water, nutrient removal from wastewater, and CO₂ sequestration, while simultaneous co-production of various biofuels is possible in a cost effective, eco-friendly, and ecologically sustainable manner.

In this study, four microalgae species including *Chlamydomonas mexicana*, *Chlorella vulgaris*, *Micractinium reisseri*, and *Scenedesmus obliquus* were screened for atrazine tolerance, and the atrazine bioaccumulation and biodegradation capability of the most tolerant *C. mexicana* was evaluated. The toxicity of atrazine on *C. mexicana* was also studied by determination of growth, chlorophyll *a* accumulation, and cellular biochemical changes.

Materials and methods

Chemicals, algal strains, culture conditions, and inoculum preparation

All chemicals used in this study were of analytical grade. Atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine] was purchased from Sigma-Aldrich (St. Louis, MO, USA), whereas methanol and other chemicals were obtained from Duksan Pure Chemicals, Ansan, Korea. The microalgal species *C. mexicana* FR751193, *C. vulgaris* FR751187, *M. reisseri* FR751194, and *S. obliquus* HM103383 were investigated in this study (Table 1). Each microalga strain was individually inoculated in a 250-mL Erlenmeyer flask containing 100 mL of Bold's basal medium (BBM) at a 10 % concentration ($V_{\text{inoculum}}/V_{\text{media}}$) (Bischoff and Bold 1963). The microalgal cells were cultivated in a shaker

incubator at 27 °C and 150 rpm, under continuous illumination of white fluorescent light of 45–50 μmol photon m⁻² s⁻¹ for 2 weeks. The microalgal suspension in BBM was adjusted to an absorbance of 1.0 at an optical density (OD) of 680 nm as measured using a spectrophotometer (Hach DR/4000, Loveland, CO, USA) and was used as the initial inoculum for further experiments.

Atrazine experiments

Initial experiments were performed to screen an atrazine-tolerant microalga strain exhibiting lowest growth inhibition after 7 days cultivation at 30 μg L⁻¹ atrazine in BBM. Experiments were conducted in each of the 500-mL serum bottles containing 300 mL of BBM inoculated with 1.5 % ($V_{\text{inoculum}}/V_{\text{BBM}}$) microalgal suspension. The bottles were incubated under white fluorescent light illumination at a light intensity of 45–50 μmol photon m⁻² s⁻¹ at 27 °C for 7 days. During the incubation, each flask was aerated with filter-sterilized air at a flow rate of 0.5 L min⁻¹ to agitate the suspension. The flasks were also hand-shaken periodically to avoid wall-growth. The selected microalga was cultivated under the same experimental conditions for 14 days in each of the serum bottles containing BBM medium added with atrazine at 0 day with the final concentrations of 10, 25, 50, and 100 μg L⁻¹, respectively. The growth rate, chlorophyll *a* content, atrazine removal capability, and biochemical changes of the microalga were determined.

Measurement of cell growth and nutrient removal, and statistics

Growth was monitored by changes in the OD₆₈₀ absorbance. A relation was obtained between the OD₆₈₀ and DCW using a standard method (APHA 1998). In brief, the microalgae suspensions were filtered through a Whatman glass fiber filter, dried overnight at 105 °C, and weighed to measure the DCW. The DCW were plotted against the corresponding OD₆₈₀ to obtain a calibration curve described by Eq. 1, which was used to convert the OD₆₈₀ of the growth into DCW.

$$\text{Dry cell weight (g L}^{-1}\text{)} = 0.3218 \times \text{OD}_{680} - 0.0139 \quad (R^2 = 0.9948) \quad (1)$$

For the chlorophyll *a* measurement, a 5-mL culture was harvested by centrifugation at 4,500×g for 10 min. The supernatant was discarded, and the pellet was re-suspended in 5 mL of 95 % methanol, incubated at 60 °C for 5 min, and centrifuged again for 10 min. The absorbance of the supernatant at 665 and 652 nm was determined with a Hach DR/4000 UV-visible spectrophotometer, and the chlorophyll *a*

Table 1 Growth inhibition percentage of *C. mexicana*, *C. vulgaris*, *M. reisseri*, and *S. obliquus* on exposure to 30 µg L⁻¹ atrazine for 7 days in BBM

Algal species	Strain ID	Accession number	DCW (g L ⁻¹) control/atrazine	Growth inhibition (%)
<i>C. mexicana</i>	YSL008	FR751193	0.373/0.315	15
<i>C. vulgaris</i>	YSL001	FR751187	0.215/0.125	41
<i>M. reisseri</i>	YSL011	FR751194	0.312/0.184	41
<i>S. obliquus</i>	YSR017	HM103383	0.321/0.140	56

DCW dry cell weight, Control without atrazine, atrazine algae cultured with 30 µg L⁻¹ atrazine

concentration of the extract was calculated using the following formula (Eq. 2), as described by Porra et al. (1989):

$$\text{Chlorophyll } a (\text{mg L}^{-1}) = 16.29 \times A_{665} - 8.54 \times A_{652} \quad (2)$$

Experiments for growth and chlorophyll *a* measurements were performed in triplicate, and the average values were reported. The data were analyzed by one-way analysis of variance with Tukey–Kramer multiple comparisons test using Graphpad Prism 5 (Motulsky 2007).

The specific growth rate (μ) was calculated by fitting the dry cell weight to an exponential function, as shown in Eq. 3:

$$\mu = \frac{\ln N_2 - \ln N_1}{t_2 - t_1} \quad (3)$$

Where N_1 and N_2 are defined as the dry cell weights at times t_1 and t_2 , respectively.

Total nitrogen (T-N) and total phosphorus (T-P) from the sample were measured using the persulfate digestion and acid persulfate digestion methods, which are equivalent to Standard Methods 4500-N C and 4500 P.B. 5, for water and wastewater, respectively (APHA 1998). Data for all experiments represent the average of three replicates.

Bioaccumulation and biodegradation of atrazine

To determine the bioaccumulation and biodegradation of atrazine, the selected microalga was treated with atrazine at concentrations of 10, 25, 50, and 100 µg L⁻¹ for 14 days. At specified intervals (6, 8, 10, and 14 days), 10 mL of alga suspension was collected, and atrazine in the cells and culture solution was individually quantified. Microalga was harvested with centrifugation at 4,500×g for 15 min. The supernatant was used for determination of the residual atrazine in the medium. The cell pellets were collected and gently washed three times using distilled water, which was collected by centrifugation as the supernatant and used for the analysis of atrazine adsorbed by the microalga. After washing with distilled water, the pellet was harvested and mixed with 3 mL

dichloromethane-methanol (1:2v/v) followed by 1 h sonication, centrifugation for 10 min at 4,500×g, and finally the supernatant was used for quantification of atrazine accumulated within the microalga cells. The atrazine residues were quantified by ultra-performance liquid chromatography (UPLC) Orbitrap mass spectrometry. Prior to UPLC analysis, atrazine from the test sample was pre-concentrated by performing online solid phase extraction with an EQUAN MAXTM system (Thermo Fisher Scientific, CA, USA). This method is based on column switching techniques and consists of a trap column and an analytical column. The trap and analytical columns used were the Hypersil Gold aQ (20 mm, 12 µm particle size) and Hypersil Gold (50 mm, 1.9 µm particle size), respectively, from Thermo Fisher Scientific. The sample delivery system consists of a CTC PAL auto sampler manufactured by CTC analytics (Zwingen, Switzerland), a six-port switching valve, and a quaternary load pump. High-resolution Orbitrap MS was performed on Exactive model with an electrospray ionization source and 50,000 resolving power (Thermo Fisher Scientific, CA, USA). Cellular accumulation of atrazine was calculated and expressed as micrograms per gram fresh weight (FW). Atrazine biodegradation by the microalga was calculated according to Eq. 4 (Liu et al. 2006):

$$P_b(\%) = (A_i - A_r - A_d - A_a - A_c) \times 100 / A_i, \quad (4)$$

where A_i is the initial quantity of atrazine, A_r is the residual quantity of atrazine in the medium, A_d is the amount of atrazine adsorbed on the microalgal cell surface, A_a is the quantity of atrazine removed by abiotic processes, and A_c is the quantity of atrazine accumulated in algal cells. A control (without alga) was run using the culture medium with atrazine, to investigate the effect of abiotic removal (e.g., light-induced decay). Data for all experiments represent the average of two replicates.

Analysis of fatty acids and carbohydrate content

The composition and content of fatty acid were determined using a modified direct transesterification method (Lepage

and Roy 1984). The fatty acid methyl esters were analyzed by gas chromatography with a flame ionization detector and a HP-INNO Wax capillary column (Agilent Technologies, USA). Helium was used as a carrier gas at 2.2 mL min⁻¹. The injection volume and split ratio were 2 μL and 45:1, respectively. Injector and detector temperatures were set at 250 °C and 275 °C, respectively. Mix RM3, Mix RM5, GLC50, and GLC70 (Supelco, USA) were used as standards. Data for fatty acid analysis represent the average of two experiments.

The carbohydrate content was determined using a phenol–sulfuric acid method (Rao and Pattabiraman 1989). Biomass (10 mg) was reconstituted in water (10 mL) to prepare a suspension of known concentration for each sample (1 mg mL⁻¹). Aliquots of sample (1 mL) were reacted with 5 mL of concentrated sulfuric acid (96 %) and 1 mL of phenol (5 %) in a water bath. The mixtures were incubated for 5 min at 90 °C. The absorbance at 490 nm was then measured using a spectrophotometer. The absorbance measurements were compared with a standard curve based on glucose. Data for carbohydrate analysis represents the average of three experiments.

Results and discussion

Effect of atrazine on the microalga growth and consequent nutrient removal

In order to select an atrazine-tolerant microalga strain, screening experiments were performed with *C. mexicana*, *C. vulgaris*, *M. reisseri*, and *S. obliquus* using BBM (30 μg L⁻¹ atrazine dose) for 7 days. *C. mexicana* showed the lowest growth inhibition compared with the other microalgae strains and was selected for further investigation (Table 1). Cell density is often used as an indicator to evaluate the toxicity of organic xenobiotic compounds on algae, as it represents an integrating parameter showing the herbicidal inhibitory effect on all cellular metabolisms (Bi et al. 2012). *C. mexicana* was treated with atrazine in the range of 10–100 μg L⁻¹ for 14 days. At 10 μg L⁻¹, atrazine had no significant inhibitory effect on the growth, while inhibition increased with the increase in atrazine concentration. The final DCW was decreased to 78 %, 67 %, and 64 % of the control at 25, 50, and 100 μg L⁻¹ atrazine, respectively (Fig. 1a).

The effect of atrazine on the microalgae depends on various factors such as algal species tested, concentration of atrazine, and end point of the experiments (Tang et al. 1998). The growth rate of *Nannochloris oculata* was inhibited by 35 % at 50 μg L⁻¹ of atrazine, while *Phaeodactylum tricoratum* was unaffected at the same concentration (Mayasich et al. 1986). It has been previously reported that atrazine significantly affects

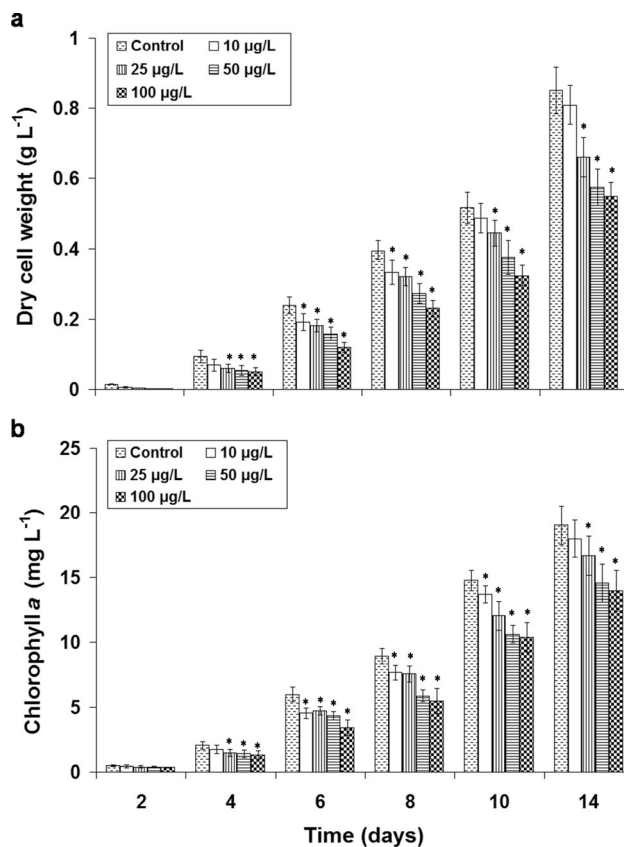


Fig. 1 a Dry cell weight and b chlorophyll a content of *C. mexicana* treated with atrazine at 0–100 μg L⁻¹ in BBM for 14 days. Values are a mean of three experiments±SEM. Asterisks indicate the significant differences between the atrazine treatments and control ($p < 0.05$)

algal metabolic patterns such as reduction of microalgal photosynthesis, chlorophyll accumulation, cell growth, and nitrogen fixation (Ramel et al. 2009). Atrazine leads to the increased generation of reactive oxygen species (ROS) such as H₂O₂ and O₂⁻ radicals (Mostafa et al. 1994; Ramel et al. 2009), which causes DNA mutation, protein denaturation, lipid peroxidation, chlorophyll bleaching, and loss of membrane integrity (Cherry and Nielsen 2004).

Effective concentration (EC₅₀), the concentration of toxicant resulting in a 50 % reduction of algal growth rate, is one of the most commonly used parameters for toxicity evaluation (USEPA 2002). A linear relationship was observed between atrazine concentration (x) and growth inhibition of *C. mexicana* (y) at 144 h in the present study, and the average 144-h EC₅₀ of atrazine was estimated to be 33.3 μg L⁻¹ according to the regression equation, $y = 0.340x + 16.25$; $R^2 = 0.997$ (Li et al. 2009). Atrazine 96-h EC₅₀ values for *Pseudokirchneriella subcapitata*, *Isochrysis galbana*, *Dunaliella tertiolecta*, *P. tricoratum*, and *Synechococcus* sp. ranged between 44.6 and 91.1 μg L⁻¹ (Weiner et al. 2004). The differential sensitivity of algal species to atrazine can be attributed to their morphology, cytology, physiology, and phylogenetics (Debelius et al. 2008).

Chlorophyll *a* is another sensitive parameter which is used to evaluate the plant response to toxic organic contaminants (Bi et al. 2012). *C. mexicana* showed a pattern of chlorophyll *a* content similar to the DCW at different atrazine concentrations (Fig. 1b). The chlorophyll *a* content was decreased to 94 %, 87 %, 76 %, and 73 % of the control at 10, 25, 50, and 100 $\mu\text{g L}^{-1}$ atrazine, respectively. Atrazine inhibits chlorophyll synthesis by retarding the formation of protoporphyrin IX, which is known to be a precursor for chlorophyll synthesis (Mofeed and Mosleha 2013). Exposure to 1 $\mu\text{g L}^{-1}$ atrazine for 7 days decreased the chlorophyll content (41–67 %) in the green algae *C. vulgaris* and *Stigeoclonium tenue* (Torres and O'Flaherty 1976).

Microalga growth at different atrazine concentrations increased the solution pH from an initial value of 6.6 to final values between 7.95 and 8.2. The photosynthetic activity of microalga consumes inorganic carbon (e.g., HCO_3^-) leading to accumulation of hydroxyl ions in the aqueous phase and an increase of solution pH (de Morais and Costa 2007). The specific growth rate of *C. mexicana* was not significantly affected with increasing atrazine concentrations and ranged between 0.48 and 0.45. Removal of inorganic nutrients was also determined after 14 days cultivation of *C. mexicana* under different concentrations of atrazine. In the culture medium, initial T-N of 52 mg L^{-1} was reduced by the microalga to 14–26 mg L^{-1} at 0–100 $\mu\text{g L}^{-1}$ atrazine, while the initial T-P of 53 mg L^{-1} was reduced to 39–50 mg L^{-1} . The potential of microalgae to uptake nutrients from contaminated wastewaters has been reported (Ji et al. 2013a, b). In this study, *C. mexicana* performed nutrient removal even in the presence of 100 $\mu\text{g L}^{-1}$ atrazine, which indicates the applicability of this alga for the bioremediation of atrazine after further investigation.

Accumulation and degradation of atrazine by microalga

Residual atrazine concentrations in medium with and without alga (control) were investigated (Fig. 2a). The concentration of atrazine in the medium with alga decreased by 41 %, 30 %, 18 %, and 20 % at 10, 25, 50, and 100 $\mu\text{g L}^{-1}$ after 14 days, respectively, while a slight reduction of atrazine (ranging between 3 % to 4 %) was observed in the control, which might be due to abiotic removal (e.g., due to photo-oxidation or other unexpected factors). At day 14, the medium with alga at all atrazine concentrations contained less amounts of atrazine than the controls (w/o alga), indicating that the atrazine was accumulated by the alga. Accumulation of atrazine over time by *C. mexicana* is shown in Fig. 2b. Atrazine accumulation was not observed for up to 2 days of cultivation, as there was no significant growth of the microalga.

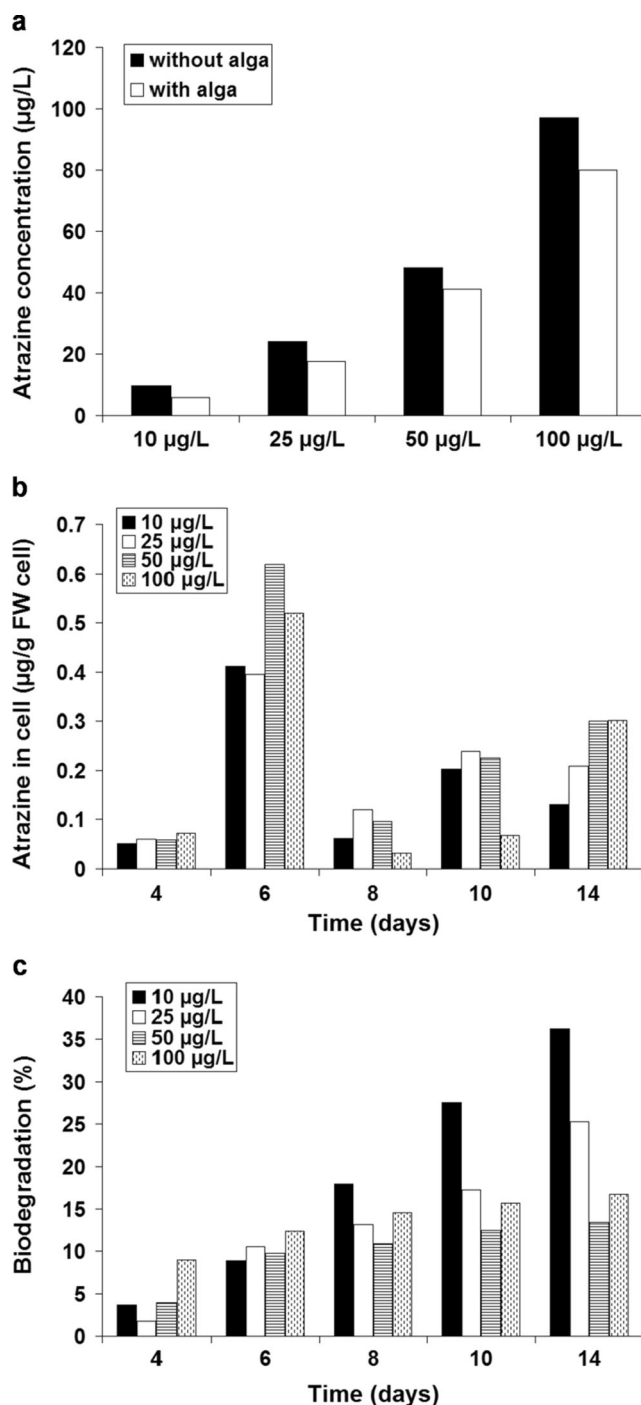


Fig. 2 a Residual atrazine in the control (without alga) and alga-incubated media after 14 days, and b, c bioaccumulation and biodegradation of atrazine by *C. mexicana* after 4, 6, 8, 10, and 14 days, at 10–100 $\mu\text{g L}^{-1}$ in BBM. Values are a mean of two experiments

However, at 4 days of cultivation, accumulations of 0.05, 0.06, 0.058, and 0.073 $\mu\text{g g}^{-1}$ FW cells were observed at 10, 25, 50, and 100 $\mu\text{g L}^{-1}$ atrazine, respectively, which further increased to 0.41, 0.39, 0.62, and 0.52 $\mu\text{g g}^{-1}$ FW cell at day 6. The higher accumulation at day 6 can be correlated with the increased growth of

the microalga (Fig. 1a). After 6 days, an unsteady accumulation of atrazine was observed at all concentrations up to 14 days.

As atrazine was unsteadily accumulated in the alga, its simultaneous biodegradation should occur during the process of accumulation. The atrazine biodegradation percentage was calculated using Eq. 4, as described in materials and methods section “Bioaccumulation and biodegradation of atrazine.” Progressive biodegradation of atrazine was observed at 10, 25, 50, and 100 $\mu\text{g L}^{-1}$, resulting in 36 %, 25 %, 14 %, and 16 % atrazine degradation after 14 days, respectively (Fig. 2c). Accumulation of atrazine by microalgae has been reported previously (Gonzalez-Barreiro et al. 2006; Baun et al. 2008), but its degradation has not been extensively investigated. In our study, although atrazine inhibited the growth of *C. mexicana*, it promoted the accumulation and simultaneous degradation of atrazine, demonstrating its capability for atrazine remediation. Toxicity of herbicides such as fluroxypyr, prometryne, and isoproturon on *Chlamydomonas reinhardtii*, and their accumulation and degradation by the microalga has been documented (Zhang et al. 2011; Bi et al. 2012; Jin et al. 2012).

Effect of atrazine on biochemical properties in *C. mexicana*

Fatty acid profile of *C. mexicana* under different atrazine concentrations

Fatty acids are a major component of the microalgal lipids, and their amount and composition is influenced by nutritional (carbon, nitrogen, phosphorous, iron, and trace metals) and environmental factors (light, temperature, and pH) (Juneja et al. 2013). The composition of fatty acids, including capric acid (C10:0), myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), and arachidic acids (C20:0), and the amounts of saturated (C10:0, C14:0, C16:0, C18:0, and C20:0), mono-unsaturated (C16:1 and C18:1), and polyunsaturated (C18:2 and C18:3) fatty acids in *C. mexicana* at different atrazine concentrations are presented in Table 2, respectively. Differential fatty acid profiles of microalga were observed at different atrazine concentrations. At 10 $\mu\text{g L}^{-1}$ of atrazine, the amounts of C10:0, C14:0, C18:0, and C20:0

increased by 79 %, 83 %, 307 %, and 69 %, respectively, compared with the control. Also, the saturated fatty acids increased by 9 %, while the polyunsaturated fatty acids decreased by 10 %. The cyanobacterium *Chroococcus* sp. reportedly undergoes a protective adaptation by decreasing the level of polyunsaturated fatty acids (membrane), which are the primary target sites for ROS induced in response to metal stress (Kumar et al. 2012). *C. mexicana* in this study might also alter its fatty acid composition by decreasing the level of polyunsaturated fatty acids as a protection mechanism against low atrazine stress (10 $\mu\text{g L}^{-1}$), with a comparable growth rate of up to 95 % of the control (Fig. 1a).

In contrast, as the concentration of atrazine was increased to 25, 50, and 100 $\mu\text{g L}^{-1}$, a progressive decrease of saturated fatty acids and an increase of unsaturated fatty acids were observed (Table 2). Atrazine causes blockage of electron transport and leads to increased generation of H_2O_2 and O_2^- radicals (ROS), which induce membrane damage and increase its fluidity (Mattoo et al. 1984). Heipieper et al. (1996) reported an increase in unsaturated fatty acid level with increasing concentration of organic pollutants in the medium. An increase of unsaturated fatty acids in the cell membrane of two algae species as a result of membrane destruction has been reported (Li et al. 2007). Thus, the increase in concentration of unsaturated fatty acids with increasing atrazine concentration might be due to higher atrazine toxicity, leading to membrane damage and, ultimately, decreased cell growth compared with the control (Fig. 1a). The quantity of C16:1 and C18:1 (monounsaturated) fatty acids increased by 400 % and 104 %, respectively (Table 2). High monounsaturated fatty acid content imparts oils with a reasonable balance of fuel properties, including ignition quality, combustion heat, cold filter plugging point, oxidative stability, viscosity, and lubricity (Rashid et al. 2008). The total fatty acids in the microalga gradually decreased from 102 to 75 mg g^{-1} with increasing atrazine concentration (0–100 $\mu\text{g L}^{-1}$).

Carbohydrate content of *C. mexicana* under different atrazine concentrations

The carbohydrates in green algae mainly come from starch (storage component) in chloroplasts and cellulose/

Table 2 Effect of atrazine concentration on the fatty acid percent composition of *C. mexicana*

Sample	C10:0	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0	SFA	MFA	PFA
Control, $\mu\text{g/L}$	1.26	1.45	34.51	0.72	0.54	5.69	33.51	20.60	1.72	39.48	6.41	54.11
10	2.26	2.66	38.19	1.57	2.20	5.72	28.49	16.01	2.90	48.21	7.29	44.5
25	1.5	0.99	36.50	0.84	1.91	6.67	28.30	21.0	2.29	43.19	7.51	49.3
50	0.91	0.57	31.89	2.96	0.45	10.24	29.81	21.69	1.48	35.3	13.2	51.5
100	0.68	0.68	27.38	3.60	1.14	11.60	29.45	23.74	1.71	31.59	15.2	53.19

SFA saturated fatty acids, MFA monounsaturated fatty acids, PFA polyunsaturated fatty acids

polysaccharides (structural components) in cell walls. Environmental factors such as nutrient starvation/limitation, salt stress, light intensity, and temperature (Carrieri et al. 2010; Branyikova et al. 2011) affect the carbohydrate content within the microalga. In addition, metabolic mode (i.e., autotrophic, heterotrophic, or mixotrophic) also affects the carbohydrate content of the microalga (Liang et al. 2009).

The carbohydrate content within *C. mexicana* at different atrazine concentrations was determined. At 10 $\mu\text{g L}^{-1}$ atrazine, the carbohydrate content (38 %) was similar to that of the control (36 %). Increasing atrazine concentration (25–100 $\mu\text{g L}^{-1}$) increased the carbohydrate content up to 47–52 %. Fournadzhieva et al. (1995) reported that the increasing concentrations of herbicide simazine up to 1 mg L^{-1} increased the carbohydrate level in *C. vulgaris*, *Scenedesmus acutus*, and *Arthrospira fusiformis*. Increasing concentrations of the herbicide glyphosate also increased the carbohydrate level in *Scenedesmus quadricauda* (El-Salam et al. 2013). Thus, an increase in the carbohydrate content of microalga with increasing atrazine concentration might be due to diversion of energy production towards the synthesis of storage compounds (carbohydrates) under atrazine stress.

Conclusion

Atrazine had a toxic effect on the microalga *C. mexicana* inhibiting its growth and chlorophyll *a* accumulation. Microalga promoted the simultaneous bioaccumulation and biodegradation of atrazine. The decrease of polyunsaturated fraction of fatty acids at lower atrazine concentrations indicated the possible protective adaptation of microalga, while the increase of unsaturated fatty acid content at higher atrazine concentrations indicated cellular damage due to high atrazine toxicity. The carbohydrate content of the microalga increased due to atrazine stress. This study demonstrated the capability of *C. mexicana* to degrade atrazine. A more specific mechanism and optimized conditions will be required for the sustainable bioremediation of atrazine contaminated streams, with simultaneous nutrient removal and biofuel feedstock generation.

Acknowledgments This work was supported by the Yonsei University Research Fund of 2013, the Mid-career Researcher Program [National Research Foundation of Korea (NRF) grant, 2013069183], the Small & Medium Business Administration (SMBA) grant C0103527 through the Academic-Industrial Common Technology Development Project, and the Eco-Innovation Project (Global-Top Project) of the Korea Ministry of Environment.

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