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RESEARCH ARTICLE

Accumulation of petroleum hydrocarbons in intracellular lipid bodies of the freshwater diatom *Synedra acus* subsp. *radians*

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Abstract The accumulation of hydrophobic compounds by phytoplankton plays a crucial role in the biogeochemical cycle of persistent organic pollutants (POPs) in aquatic environments. We studied the accumulation of polycyclic aromatic hydrocarbons (PAHs) in the freshwater diatom *Synedra acus* subsp. *radians* during its cultivation with crude oil hydrocarbons, using epifluorescent and laser confocal microscopy as well as gas chromatography–mass spectrometry (GC/MS) analysis. Our results revealed that in the presence of crude oil or an extract of a crude oil/*n*-hexane solution (light oil), *S. acus* subsp. *radians* accumulated PAHs in its lipid bodies. During cultivation in the presence of a crude oil/*n*-hexane solution, the cells selectively accumulated C12–C18 alkanes, with a preference for C15 and C16 homologues. The length of *n*-alkane hydrocarbon chains accumulated in cells was similar to the acyl chains of fatty acids of the diatom. We therefore suggest that the insertion of *n*-alkanes into the membrane lipid bilayer promotes the transmembrane transport of PAH in diatoms. Our results confirm the hypothesis that diatoms play a role in the elimination of hydrophobic hydrocarbons from aquatic systems.

Keywords PAH · Lipid bodies · Accumulation · Diatom · Lake Baikal · *Synedra acus* subsp. *radians*

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Introduction

Phytoplanktons play a key role in the accumulation of hydrocarbons, including polycyclic aromatic hydrocarbons (PAHs), entering aquatic ecosystems (Lei et al. 2007; Hong et al. 2008; Nizzetto et al. 2012). By accumulation and transformation of hydrophobic hydrocarbons, phytoplankton participates in the transfer of these substances to higher trophic levels and the involvement into the carbon cycle, which may have harmful effects on the higher consumers in the food web (Kingston 2002; Soto et al. 2014) and generally impact ecosystem functioning.

To determine the pathway of hydrocarbons in the aquatic system, an evaluation of the bioconcentration factors (*BCF*s) for these compounds is crucial. The bioconcentration level depends on the physical and chemical properties of hydrophobic compounds (Swackhamer and Skoglund 1993; Gerofke et al. 2005; Ko et al. 2012), cell surface hydrophobicity (Katagi 2010), and the qualitative and quantitative lipid content of the cells (Berglund et al. 2001).

Mechanisms of hydrocarbon accumulation and biodegradation were mainly studied using prokaryotic organisms (Watkinson and Morgan 1990) with biosurfactant activity (Beal and Betts 2000; Bouchez-Naïtali and Vandecasteele 2008). Based on kinetic analyses, active and passive transmembrane [C^{14}]phenanthrene transport has been found in the bacterium *Anthrobacter* sp. (Kallimanis et al. 2007). A temperature-independent benzo(a)pyrene transport has been shown in the filamentous fungus *Fusarium solari* (Verdin et al. 2005). Epifluorescence microscopic studies have revealed passive diffusion of benzo(a)pyrene into the lipid bodies of *F. solari* cells. The authors proposed that the mechanism of passive benzo(a)pyrene uptake can be explained by the properties of destabilization of the lipid mosaic by lipophilic molecules. Uptake and accumulation of phenanthrene in lipid vesicles using two-photon excitation microscopy (TPEM) were demonstrated

in the filamentous fungus *Pythium ultimum* (Furuno et al. 2012). The location and the aggregation of benzo(a)pyrene in the lipid bodies of *Chlorella* sp. were characterized with fluorescence confocal microscopy and fluorescence lifetime imaging using the phasor approach (Subashchandrabose et al. 2014).

To the best of our knowledge, there are no studies examining the role of lipid bodies in diatoms during PAH accumulation. However, in freshwater ecosystems used as drinking water sources, this aspect is of vital importance. For example, in Lake Baikal, Siberia, the world's largest freshwater reservoir, diatoms contribute significantly to lake primary production (Popovskaya 2000). Through accumulation of organic hydrophobic compounds along with bacteria capable of degrading hydrocarbons, diatoms take part in the bioremediation of the surface water of the lake (Pavlova, et al., 2008; Gorshkov et al. 2010) and transport this compounds upwards in the food chain of Baikal (Kucklick et al. 1996), including sites of natural oil seeps (Khlystov et al. 2007; Kontorovich et al. 2007).

Freshwater diatom *Synedra acus* subsp. *radians* (Kütz.) Skabitschevsky is one of the dominant species in the phytoplankton of Lake Baikal (Popovskaya et al. 2016) and can serve as a good model organism for ecological and ecotoxicological studies. The main aim of this work was to investigate the accumulation and storage of petroleum hydrocarbons by this freshwater diatom. Our specific objectives were to (1) determine the concentrations crude oil and light oil causing inhibitory effects on diatom growth, (2) quantify the kinetics of PAH accumulation in the diatom during cultivation with light oil, and (3) evaluate the importance of *n*-alkanes for PAH accumulation in the diatom's lipid bodies. We address these objectives through detailed chemical analyses of PAHs and *n*-alkanes using gas chromatography–mass spectrometry (GC/MS) and a combination of epifluorescent and scanning confocal microscopy to characterize PAH accumulation in intracellular lipid bodies.

Materials and methods

Preparation of crude oil and light oil solutions

Crude oil was supplied by the Angarsk refinery (deposits of western Siberia) and contained 15.3 % of *n*-alkanes and 1.2 % of PAHs. To remove bacterial contaminants, the crude oil sample was filtered through a polycarbonate filter with a pore diameter of 0.2 μm (Millipore, USA). Light oil was obtained by mixing crude oil with *n*-hexane (1:4 *m*/*v*) using sonication at 40 kHz for 5 min (Ferroplast Medical ultrasonic bath, Russia), followed by separation of the *n*-hexane supernatant from the lighter hydrocarbon fraction by centrifugation at 1000*g* for 3 min. Quantitative analysis of the residual showed that the mass of the heavy asphaltene fraction was gravimetrically quantified after solvent evaporation to constant weight (Capelli et al. 2001) which did not exceed 6 %.

Experimental growth conditions for the diatom *S. acus* subsp. *radians*

S. acus subsp. *radians* was isolated from a natural population sampled in Listvenichniy bay, Lake Baikal, in 2010, during the season of its dominance in the phytoplankton. An axenic culture of *S. acus* subsp. *radians* (Shishlyannikov et al. 2011) was grown in 1-l Erlenmeyer flasks with 250 ml of sterile DM medium (Beakes et al. 1988) of the following composition (concentration given in mg l^{-1}: $Ca(NO_3)_2 \cdot 4H_2O$, 20; KH_2PO_4, 12.4; $MgSO_4 \cdot 7H_2O$, 25; $NaHCO_3$, 16; Na_2EDTA, 2.25; H_3BO_3, 2.48; $MnCl_2 \cdot 4H_2O$, 1.39; $(NH4)_6Mo_7O_{24} \cdot 4H_2O$, 1.0; $Na_2SiO_3 \cdot 9\ H_2O$ 11.4; cyanocobalamin, 0.04; thiamine hydrochloride, 0.04; biotin, 0.04). Crude oil or light oil at concentrations of 10, 50, 100, and 250 mg l^{-1} of the medium was added to the axenic culture during the exponential growth phase (6000–7000 cells ml^{-1}). The diatom was grown for 13 days at 6 °C and a 14:10-h light/dark cycle. Samples were shaken occasionally. Each experiment was performed in triplicate.

For determination of the maximum cell division rates in the presence crude oil and light oil, subsamples for cell counting (1 ml) were taken at approximately the same time every day. The abundance of diatoms (cells ml^{-1}) was estimated by enumerating cells on a glass slide with an epifluorescence microscope Axiovert 200 (Carl Zeiss, Germany). Maximum cell division rates (μ) were calculated using the following formula:

$$\mu = N_{t2}/N_{t1}/(t_2 – t_1) \quad (1)$$

where N_{t2} and N_{t1} are cell numbers in 1 ml at times t_2 and t_1 (Thomas and Dodson 1974). We used the average value of three successive cell counts in the calculations.

Determination of PAHs and *n*-alkanes

We quantified the concentrations of PAHs and *n*-alkanes in the cells and supernatant separately. Before the chemical analysis, 50 μl of the surrogate internal standards (naphthalene-d_8, acenaphthene-d_{10}, phenanthrene-d_{10}, chrysene-d_{12}, pyrelene-d_{12}, 5 ng μl^{-1} each, in acetonitrile) and squalane (130 ng μl^{-1} dissolved in *n*-hexane) were added to the fractions containing cells or supernatant. Using ultra-sonic treatment, PAHs and *n*-alkanes were extracted from the culture medium with *n*-hexane (20 ml, twice) and from wet cell biomass (weight sample ~25 mg) with *n*-hexane/acetone mixture (1:1 *v*/*v*, 10 ml, twice). Obtained extracts were dried under anhydrous K_2SO_4, centrifuged as described above, and aliquots of the part of extract (0.5 ml) were taken for determination of naphthalene's. Then, the residual extracts were concentrated to 1–2 ml with a rotor evaporator at 40 °C (IKA, Germany). The final extracts were reduced to a volume of about 0.5 ml under argon stream and analyzed using a gas chromatograph (Agilent GC system 6890)

equipped with a HT-8 capillary column (30 m length, 0.25 mm internal diameter, and 0.25 μm film thickness, SGE, Austin, TX, USA) with an autosampler, a split/splitless injector, and coupled with a mass selective detector (Agilent MSD 5973 Network) with an electron impact mode at 70 eV. Helium was used as carrier gas at a constant flow of 1.0 ml min^{-1} with injector, quadrupole detector, and ion source temperatures of 290, 150, and 230 °C, respectively. The extracts (2 μl) were injected in splitless mode into the column. The oven temperature was programmed to increase from 80 to 310 °C at a rate of 7 °C min^{-1} and kept constant for 3 min. Peaks of PAHs and *n*-alkanes were detected in the selected ion monitoring mode. Selected recording ions were as follows: *m*/*z* values of 128, 136, 142, 152, 154, 164, 166, 178, 188, 202, 228, 240, 252, 264, 276, and 278 (for PAHs), and 57, 71, and 183 (for *n*-alkanes). PAH and *n*-alkane peaks were identified by their relative retention times using the external standard (Polynuclear Aromatic Hydrocarbons Mix, Supelco, USA), chromatograms of light oil, and mass-spectrums *n*-alkanes.

The quantification of PAHs was based on the internal standard method using deuterated surrogate internal standards. The calibration was done for each PAH by internal calibration of certified mixture PAHs. Five concentrations of calibration standard in the range expected in sample were analyzed to obtain a linear curve fit with R^2 value of 0.997. Surrogate standard was used to monitor losses during extraction. Consistent recovery of PAHs (≥90 %) was observed during analysis. The reliability of the calibration was checked by injecting known standard and solvent blanks into the column. Values below the mean plus three standard deviations of the blank values were considered below limit of quantification (LOQ). Instrument limit of detection (ILOQ) was calculated as three times the chromatogram baseline noise level and for PAHs is 10–20 ng g^{-1} of wet weight (ww).

Determination of PAH bioconcentration factor

PAH bioconcentration factors were calculated according to the following formula (Sijm et al. 1995):

$$BCF_{\mathrm{ww}} = \frac{C_{\mathrm{cells}}}{C_{\mathrm{water}}} \quad (2)$$

where C_{cells} is the PAH concentration in diatom cells after centrifugation (mg kg^{-1} of wet weight) and C_{water} is the PAH concentration in the supernatant of the culture medium (mg kg^{-1}).

Epifluorescence microscopy for detection of PAHs in lipid bodies

The intracellular uptake of PAHs in lipid bodies of diatom was monitored by epifluorescent microscopy during cultivation of diatom in the presence of petroleum hydrocarbons (Verdin et al. 2005). Ten microliters of Nile red dye (Sigma, USA, 2 mg ml^{-1} acetone solution) was added to 1 ml of the cell culture at different growth phases (Shishlyannikov et al. 2014), incubated for 5 min in the dark, and analyzed using an epifluorescence microscope Axiovert 200 (Carl Zeiss, Germany) equipped with a mercury lamp Osram HBO 50 W/AC, Penguin 600CL digital camera (Pixera Corp., USA) and the software "AxioSet" (Carl Zeiss, Germany). Fluorescence analysis was carried out at the excitement wavelengths of 365 nm for PAHs and 450–490 nm for Nile red and at the emission wavelengths of 420 and 515 nm, respectively.

Laser confocal microscopy for localization of PAHs in lipid bodies

The intracellular localization of PAH in lipid bodies was determined using laser confocal microscopy (Furuno et al. 2012).

S. acus subsp. *radians* cells were isolated from the culture medium by centrifugation at 1000*g* for 10 min and washed twice with 400 μl of sterile DM medium. The cells were then fixed with 2 % paraformaldehyde solution (Sigma, USA) for 15 min. Subsequently, diatom cells were placed into ProLong® Gold antifade reagent (Life Technologies, USA) and observed with a laser confocal microscope LSM 710 (Carl Zeiss, Germany) equipped with a Plan-Apochromat 63×/1.40 Oil DIC M27 objective lens (Carl Zeiss, Germany).

The fluorescence excitation and emission wavelengths for lipid bodies were 405 and 420–550 nm; those for chloroplast fluorescence were 561 and 650–723 nm, respectively. Microscopic images were processed using the ZEN 2010 program (Carl Zeiss, Germany).

Statistical analysis

The experimental values for maximum cell division rates of *S. acus* subsp. *radians* to crude oil and light oil hydrocarbons and data for PAH accumulation by *S. acus* subsp. *radians* cultivated in the presence of light oil were calculated as average $\bar{x}$ from two replicates with confidence interval Δx. The 95 % confidence interval values Δx for $\bar{x}$ were derived from Student's *t* distribution with symmetric probability distribution using the formula (Smagunova and Karpukova 2008):

$$\Delta x_1 = \Delta x_2 = \Delta = \frac{t(\alpha, f)\cdot S}{\sqrt{n}} \quad (3)$$

where S is the standard deviation (SD) of the concentration values, average values $\bar{x}$, $\alpha = 1 - P = 0.05$ (P is the level of confidence of 95 %), n is the number of measurements, $f = n - 1 = 2$ is the degree of freedom to calculate the S values, and $t(\alpha, f)$ is the t table value.

Results and discussion

Toxic effect of crude oil on *S. acus* subsp. *radians* growth

The toxic effect of crude oil hydrocarbons on the growth of *S. acus* subsp. *radians* was studied using crude oil (Fig. 1) and an extract of a crude oil/*n*-hexane solution (light oil) without fractions of high molecular asphaltene hydrocarbons (Fig. 1).

We observed no growth inhibition of *S. acus* subsp. *radians* at the crude oil concentration of 10 mg l^{-1}. However, cell growth rates decreased by 1.5 times when the concentration was at 50 mg l^{-1}. A further increase in the concentration resulted in cell death (Fig. 1). The results obtained for the light oil inhibition were similar to the crude oil inhibition of diatom cell growth.

The relative standard deviation (RSD) values describing the precision of the estimates of maximum division rates in the presence of crude oil were 8 % (for 0–50 mg l^{-1} concentration) and 31 % (for 100–250 mg l^{-1} concentration). A significant precision decline of the results obtained in conditions of relatively high hydrocarbon concentrations (100–250 mg l^{-1}) in the medium was noted. The RSD values for light oil concentrations were estimated as 13 % (for 0–100 mg l^{-1} concentration) and 22 % (for 250 mg l^{-1} concentration).

The toxic effect of crude oil and light oil on the growth of freshwater and marine microalgae depends on the microalgae species, the crude oil type, the concentrations of hydrocarbons in the aqueous phase, and the time and temperature of exposure (Corner 1978, Ozhan et al. 2014). Among the hydrocarbons in crude oil, PAHs are the most toxic ones for different representatives of planktonic and benthic algae (Djomo et al. 2004; Gamila and Ibrahim 2004; Wang et al. 2008; Ozhan and Bargu 2014; Croxton et al. 2015). Previous studies have revealed contradicting effects on the influence of crude oil hydrocarbons on different species of microalgae. In particular, hydrocarbons in the aqueous phase at a concentration of 1 mg l^{-1} inhibited the growth of the arctic diatom *Melosira moniliformis* and stimulated the growth of *Ditylum brightwellii* (Mironov and Lanskaja 1967). However, the rate of cell division in *D. brightwellii* declined at a crude oil concentration of 10 mg l^{-1}. In addition, recent study has shown that the two diatom species, *D. brightwellii* and *Chaetoceros socialis*, demonstrate higher tolerance to crude oil than the three dinoflagellates species, *Pyrocystis lunula*, *Scrippsiella trochoidea*, and *Heterocapsa triquetra*, and the larger species of phytoplankton were more tolerant to crude oil than the smaller ones (Ozhan et al. 2014). Our results show that inhibition of *S. acus* subsp. *radians* takes place at a crude oil hydrocarbon concentration between 10.0 and 50.0 mg l^{-1}, which corresponds to a concentration toxic for the most known microalgae (Anderson et al. 1974; Kusk 1981; Fabregas et al. 1984; Tukaj 1987) and cyanobacteria (Obaidy et al. 2014).

Localization and identification of PAHs in lipid bodies

Blue fluorescence of 1–2-µm-sized oval formations was observed by epifluorescence microscopy in the diatom cytoplasm after day 5 of growth (exponential phase) in the presence of 10 mg l^{-1} of crude oil or light oil (PAH concentration in the medium was 120 µg l^{-1}). These formations were located in the center and in the periphery of the cells, reaching a size of 4–5 µm on day 13 (stationary growth phase) (Fig. 2a). Control experiments with diatoms cultivated without crude oil hydrocarbons resulted in no fluorescence (Fig. 2b).

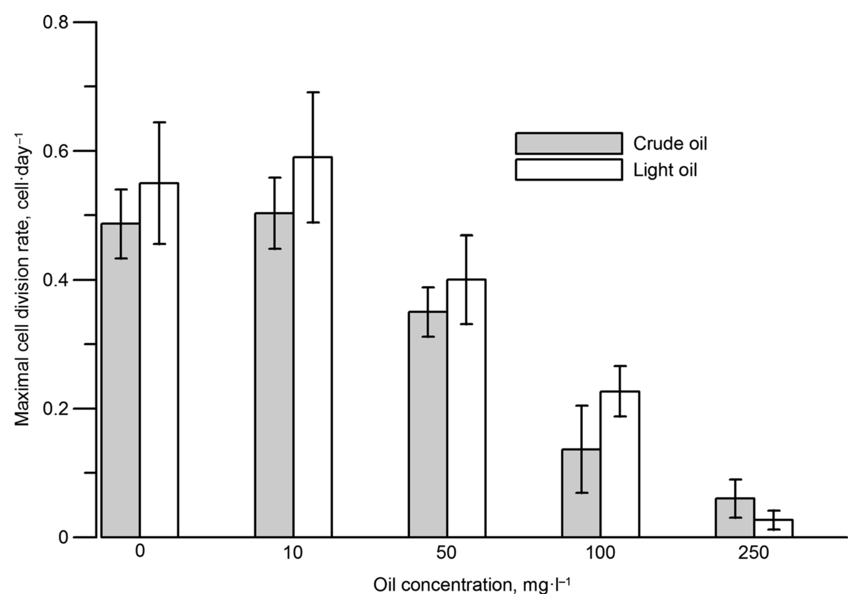

Fig. 1 Effect of crude oil and light oil on *S. acus* subsp. *radians*

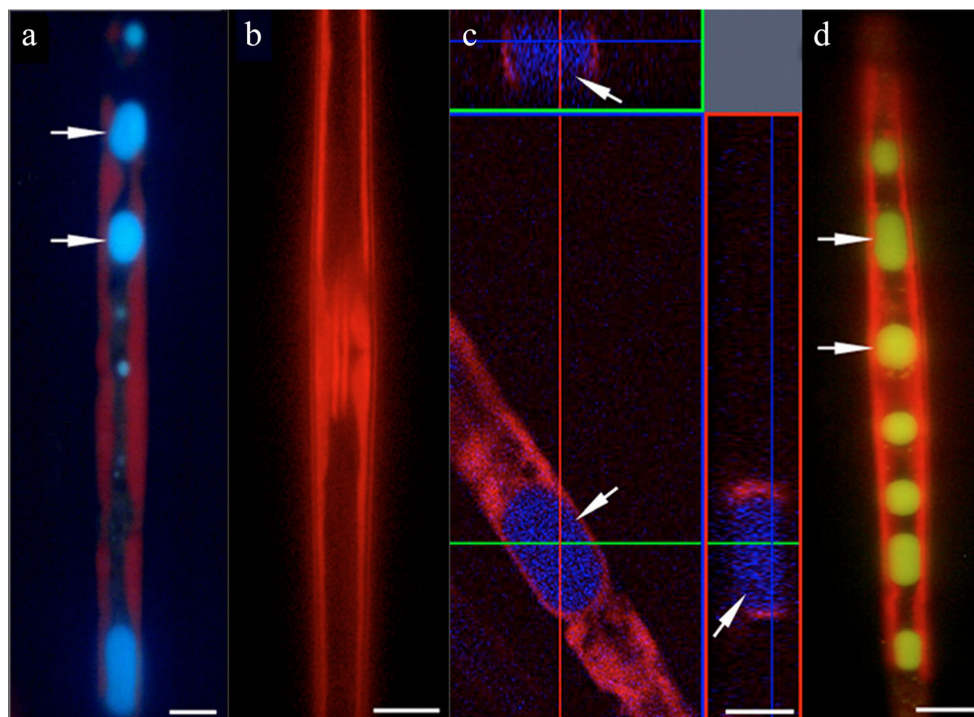

Fig. 2 PAH accumulation in lipid bodies of *S. acus* subsp. *radians* after 13 days of cultivation. **a**, **b**, **d** Epifluorescence microscopy. **c** Laser confocal microscopy (orthogonal slice view). **a** In the presence of crude oil (PAH concentration 120 μg l^{-1}). **b** Without crude oil (control). **c** In the presence of light oil (PAH concentration 120 μg l^{-1}). **d** Lipid bodies stained with Nile red (after cultivation of diatom in the presence of light oil). *White arrows* show lipid bodies. Chloroplast—*red*, PAHs—*blue*, stained lipid bodies—*yellow*. *Scale bar*—5 μm

To confirm the intracellular localization of PAHs, we used laser confocal microscopy (Furuno et al. 2012). Under these conditions, the image (Fig. 2c) clearly demonstrated the spatial location of blue-colored fluorescent vesicles within limits of cell (between two chloroplasts within cell limits).

The nature of the cellular compartment involved in PAH storage investigated using Nile red dye is specific to neutral lipids (Greenspan et al. 1985). We used this dye to identify the lipid nature of orange-red formations found in cells growing in the presence of light oil (Fig. 2d). Nile red has also been used for the detection of benzo(a)pyrene accumulation in lipid bodies of *Chlorella* sp. (Subashchandrabose et al. 2014).

PAHs are the main components of crude oil and are capable to fluoresce (Konstantinova-Schlesinger 1961). PAHs are excited generally in the 200–400 nm range and are also strongly fluoresce. Multicomponent mixtures of PAHs have spectral overlap excitation and emission wavelengths (Patra 2003). The data obtained by laser confocal microscopy (Fig. 3) shows that the fluorescence of lipid bodies in *S. acus* subsp. *radians* ranged from 450 to 550 nm, which is a characteristic

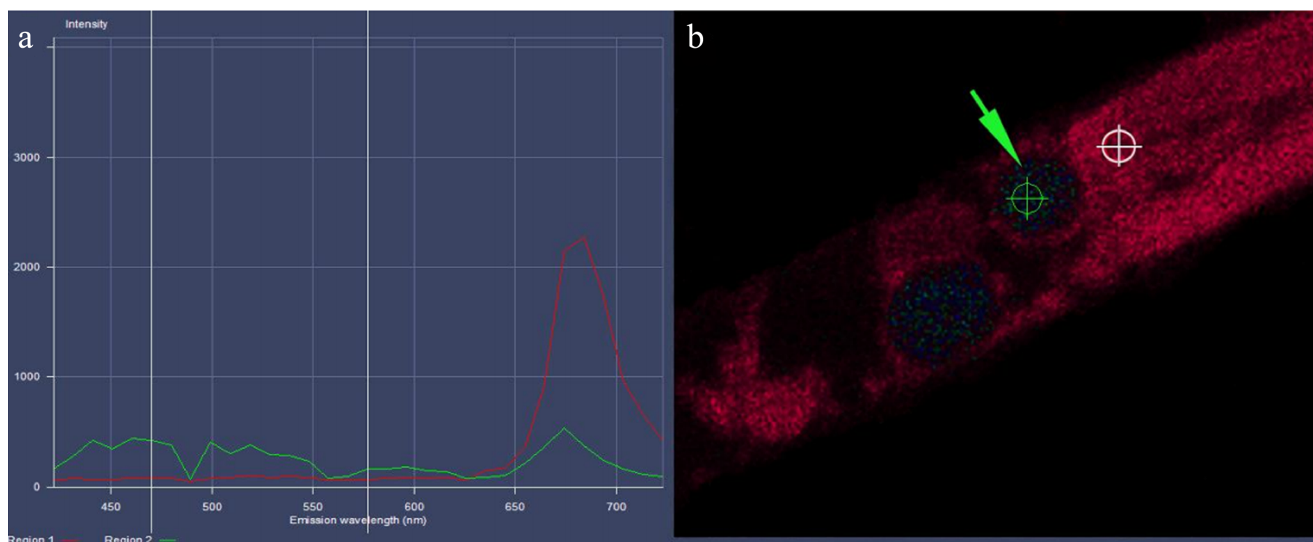

Fig. 3 Laser confocal microscopy of *S. acus* subsp. *radians* after 5 days in the presence of light oil (10 mg l^{-1}). **a** Fluorescence spectrum of lipid bodies (*green line*) and chloroplast (*red line*). **b** 2D optical section; *green arrow* shows a lipid body. The scanning emission spectrum zones are shown with marks (*green cross*—lipid body, *white cross*—chloroplast)

for PAH emission (Warner et al. 1977). This observation leads to the conclusion that diatoms accumulated PAHs in intracellular lipid bodies.

It may be assumed that PAHs also accumulated in the chloroplast because the membranes of chloroplasts have the highest contents of lipids. Nevertheless, we did not identify the emission spectrum of PAH in diatom chloroplasts. This was probably caused by the wide spectrum of autofluorescent substances found in chloroplasts, and thus, it is difficult to distinguish PAHs' fluorescence from additional molecules.

Assessment of bioconcentration factors for PAHs in *S. acus* subsp. *radians*

Crude oil usage for the estimation of bioconcentration factors (BCF_{ww}) can lead to an incorrect interpretation of results due to the presence of an insoluble asphaltene fraction in the water-organic phase of the culture medium, which is the concurrent adsorption site for hydrocarbons dissolved in the medium. Therefore, we used the light oil in the experiments of BCF_{ww} determination for PAHs in *S. acus* subsp. *radians*. We identified 17 PAHs in light oil using GC/MS techniques. Twelve PAHs are listed in Table 1; the remaining five compounds were present only in minimal quantities (below 1.5 %).

In rapidly growing diatoms, PAH concentrations in cells are reduced by the accumulation of new biomass, a phenomenon known as growth dilution (Swackhamer and Skoglund 1993), so BCF_{ww} values for PAHs are best determined in conditions of slow growth. Therefore, analysis of PAH concentration factor was performed at pre-stationary growth phase (0.09 cells day^{-1}) and in the asphaltene-free light oil treatment.

Total PAH concentration in the diatom biomass cultivated in medium containing 10 mg l^{-1} of light oil was 45 μg g^{-1} wet weight (ww) after the first incubation day at the pre-stationary growth phase and did not change until day 5 (Table 1). We used these data for an assessment of factors influencing water-diatom partitioning.

The partitioning coefficient becomes invariant (reaches a steady state) theoretically at the point at which the fugacity ratio equals 1 (Mackay 1982). If bioconcentration of hydrophobic compounds in microalgae primarily concerns partitioning into the lipid fraction (Swackhamer and Skoglund 1993), it can be assumed to be proportional to K_{ow} (octanol-water partition coefficient):

$$BCF_{\mathrm{PAHs}} \approx \alpha \cdot K_{ow}, \tag{4}$$

where α is dependent on the lipid content and lipid class and therefore depends on different species and their physiological states.

BCF_{PAH} values were calculated from diatom and aqueous phase measurements (Fig. 4). Plots for logarithmic dependencies of BCF_{PAH} on K_{ow} (for $BCF_{PAH} < 5$) had maxima at $\log K_{ow} = 4.5$ to 5.0. BCF_{PAH} values for benzo(a)anthracene (11) and chrysene (12) ($\log K_{ow}$ 5.6 and 5.7, respectively) were not taken into consideration due to lower solubility of these compounds in water and their high molecular weights which likely prevent their transport through the diatom plasmalemma (Dimitrov et al. 2002). Figure 4b shows that α values $BCF_{PAH} < 5$ are about 1.3 times higher after day 5 compared

Table 1 PAH accumulation by *S. acus* subsp. *radians* grown with light oil (at concentration—10 mg l^{-1})

Compounds	PAHs concentration in medium, μg l^{-1}	PAHs concentration in cells, ($x_{cp.} \pm \Delta$), μg g^{-1} ww	
		Growth duration, days	
		1	5
Naphthalene (1[a])	30	15.0 ± 2.0	15.4 ± 1.7
Acenaphthene (2)	5	2.00 ± 0.20	2.20 ± 0.21
Acenaphthylene (3)	0.5	0.52 ± 0.06	0.77 ± 0.09
Fluorene (4)	8	2.7 ± 0.3	3.1 ± 0.4
Phenanthrene (5)	16	11.0 ± 1.0	13 ± 1.4
Fluoranthene (6)	0.4	1.12 ± 0.12	1.65 ± 0.24
Pyrene (7)	0.6	0.92 ± 0.12	1.52 ± 0.24
2-Methylnaphthalene (8)	29	6.7 ± 0.8	5.0 ± 0.6
1-Methylnaphthalene (9)	26	4.7 ± 0.5	3.4 ± 0.4
Anthracene (10)	0.5	0.28 ± 0.03	0.37 ± 0.04
Benz(a)anthracene (11)	0.3	0.052 ± 0.010	0.100 ± 0.011
Chrysene (12)	0.9	0.14 ± 0.02	0.22 ± 0.03
Σ total PAH	120	45.0 ± 2.0	45.0 ± 2.0

[a] Individual PAH numbers were used to construct $\log BCF_{ww} = f \log K_{ow}$ dependence (see Fig. 4)

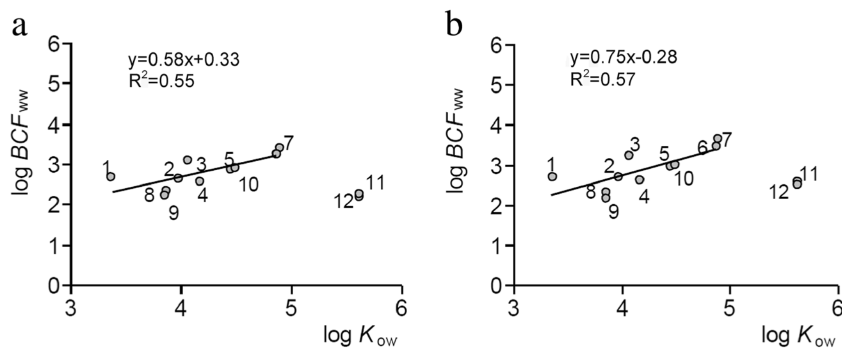

Fig. 4 LogBCF_{ww} versus logK_{ow} for *S. acus* subsp. *radians* cultivated in the presence of light oil (10 mg l^{-1}). **a** After 1 day. **b** After 5 days. The logBCF_{ww} = *f*(logK_{ow}) plots were calculated for PAHs with logK_{ow} <5. The plots were made and the correlation coefficients *R* were calculated using all points with the exception of 11 and 12

to day 1 when they were cultivated in the presence of light oil (0.75 and 0.58, respectively). This finding can be explained by an increase of the lipid impact on the accumulation of PAHs by the diatom during cultivation. The lipid content of *S. acus* subsp. *radians* is composed of fatty acids in the lipid membrane bilayer and intracellular lipid bodies (Shishlyannikov et al. 2014). PAH accumulation in lipid bodies observed by cytochemical methods (Fig. 2c) supports this hypothesis. The importance of the lipid content in bioaccumulation of hydrophobic compounds by aquatic organisms has been ascribed recently (Katagi 2010; Croxton et al. 2015). According to the previously published data by Croxton et al. (2015), increasing the lipid mean value in diatom for all PAH-exposed treatments was significantly higher than control treatments. It was suggested that “more energy to maintain cell growth or for diluting the PAH compound within a larger cellular lipid pool” (Croxton et al. 2015). In the present study, we did not observe similar results. This is probably related to the differences of the concentrations of individual PAHs both in culture mediums and diatom’s species. In addition, we used light oil, which included both PAHs and *n*-alkanes, whereas Croxton et al. (2015) used the individual PAHs.

n-alkane accumulation by *S. acus* subsp. *radians*

The composition of *n*-alkanes found in *S. acus* subsp. *radians* cells after cultivation in the presence of light oil was significantly different compared to the composition of *n*-alkanes in the culture medium (Fig. 5a). Several *n*-alkanes with a hydrocarbon chain length from C12 to C18 were accumulated, with a preference for *n*-alkanes with a chain length of C15 and C16 (Fig. 5b). Patterns of *n*-alkane or PAHs in the control groups were not detected. It should be noted that *n*-alkanes that accumulated in the diatom cells have a chain length similar to that of acyl chains of fatty acids in the lipid component of *S. acus* subsp. *radians* (Shishlyannikov et al. 2014). The same selectivity in *n*-alkane accumulation with a preference for chain lengths of C13 to C16 was observed when the seawater diatom *Cyclotella cryptica* was exposed to crude oil (Karydis 1980).

Sikkema et al. (1995) showed that the accumulation of lipophilic substances and their interaction with the phospholipid layer of the cytoplasmic membrane caused its modification and changed cytoplasmic membrane functioning. Since hydrocarbons are non-polar compounds, they can be incorporated into the membrane and enter the cell by passive diffusion. PAH and *n*-alkane insertion into the membrane bilayer was demonstrated using artificial vesicles where *n*-alkanes penetrated into the vesicles by intercalating with chains of fatty acids of similar lengths (Hunt and Tipping 1978;

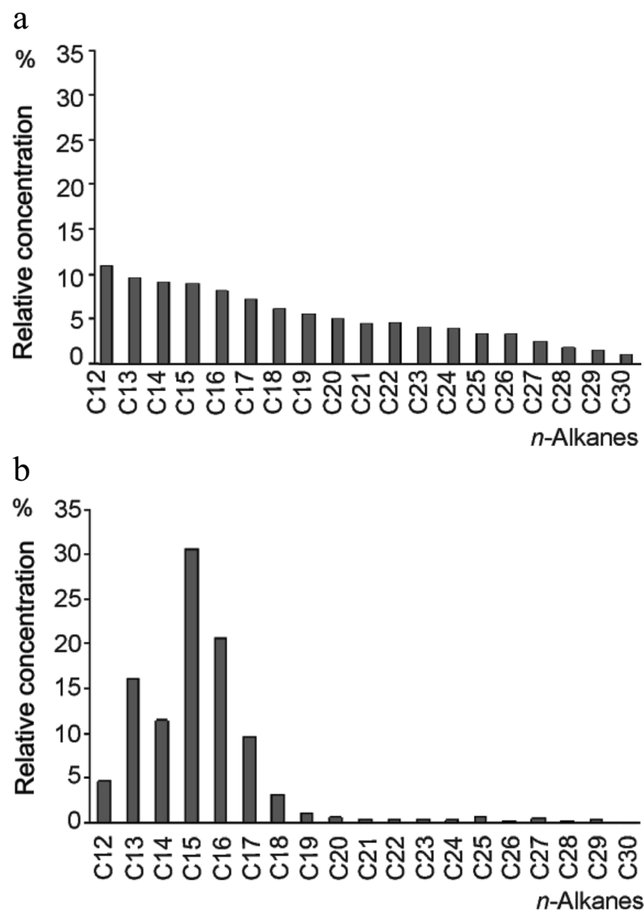

Fig. 5 Relative partitioning of *n*-alkanes between culture medium (**a**) and *S. acus* subsp. *radians* cells (**b**) after 5 days of cultivation in the presence of light oil

Castelli et al. 2002). Studies on gene expression and transcriptomics responses of the diatom *Thalassiosira pseudonana* exposed to PAHs have shown that PAHs inhibited the formation of new silica valves to decrease diatom growth rates (Bopp and Lettieri 2007, Carvalho et al. 2011). According to these works, PAHs impair the lipid metabolism and silica shell formation. The growth inhibition and the subsequent death of *S. acus* subsp. *radians* at a PAH concentration higher than 600 μg l^{-1} (at concentration of light oil in medium—50 mg l^{-1}) also can be explained by an increasing negative effect of PAH accumulation in lipid bodies on diatom metabolism and morphogenesis of new cell valves. We propose that *n*-alkanes with a chain length similar to acyl chains of fatty acids in lipid bodies of diatoms inserted into the plasmalemma bilayer contribute to the passive hydrocarbon diffusion into the cell, including PAHs which are accumulated in the lipid bodies, leading to an inhibition of the intracellular metabolism of the diatom.

Conclusion

In this work, we demonstrate that intracellular accumulation of PAHs into lipid bodies of *S. acus* subsp. *radians* takes places during cell growth in the presence of crude oil hydrocarbons. In the process of intracellular PAH accumulation, diatom cells selectively accumulate *n*-alkanes with a chain length from C12 to C18 from the culture medium, with a preference for the chain lengths C15 and C16. The hydrocarbon chain length of accumulated *n*-alkanes is similar to that of the acyl chains of fatty acids in the lipid content of diatom. We therefore suggest that *n*-alkanes contribute to PAH transmembrane transport and that PAH transport from the aquatic environment into the cell and accumulation in lipid bodies is a universal process. Our study shows that diatoms can participate in PAH bioaccumulation with the subsequent removal of these substances from the surface layer water by precipitation of the cells or incorporation in the trophic web in crude oil-polluted sites.

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