J Appl Phycol (2015) 27:1443–1451
DOI 10.1007/s10811-014-0477-1

Trace concentrations of iron nanoparticles cause overproduction of biomass and lipids during cultivation of cyanobacteria and microalgae

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Received: 11 August 2014 / Revised and accepted: 24 November 2014 / Published online: 12 December 2014

Abstract Cultivation in Zehnder medium containing 5.1 mg L^{-1} zero-valent iron nanoparticles (nZVI) boosted the growth of the green algae *Desmodesmus subspicatus*, *Dunaliella salina*, *Parachlorella kessleri* and *Raphidocelis subcapitata* and the eustigmatophycean algae *Nannochloropsis limnetica* and *Trachydiscus minutus*. In the cyanobacterium *Arthrospira maxima*, growth stimulation occurred at 1.7–5.1 mg L^{-1} nZVI. In all studied microorganisms, 5.1 mg L^{-1} nZVI strongly enhanced lipid accumulation, decreased the content of saturated and monounsaturated fatty acids with the exception of palmitoleic acid and increased the content of polyunsaturated fatty acids in cells. The nZVI particles may provide a suitable source of iron causing increased cell growth and induce metabolic changes resulting in higher lipid production and changes in fatty acid (FA) composition. Altered lipid synthesis may reflect the oxidative action of nZVI. Further research may contribute to optimizing the economical production of oils from oleaginous microorganisms and help clarify the mechanism of nZVI action.

Keywords Zero-valent iron · Nanoparticles · Microalgae · Cyanobacterium · Lipid profile

Electronic supplementary material The online version of this article (doi:10.1007/s10811-014-0477-1) contains supplementary material, which is available to authorized users.

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Introduction

Nanotechnologies are rapidly emerging in many fields of medicine, industry or environmental sustainability. Nanomaterials have physical and chemical characteristics such as chemical reactivity; electric, magnetic and optical properties; and biological activity that differ from their bulk forms (Crane and Scott 2012). The ever-extending range of applications of nanomaterials brings questions dealing with the problem of their overall impact on the environment. The main problems to have been discussed are associated with their toxicity against exposed organisms (Nel et al. 2006). However, some recent studies have also dealt with the possible positive effects of these materials on cell growth or metabolic activity (Hong et al. 2005; Kadar et al. 2012; Mahajan et al. 2011).

Due to their size (<100 nm), engineered nanoparticles can readily interact with cell surface and even penetrate into cytosol (Verma and Stellacci 2010). The considerable bioavailability of nanoparticles (Johnston et al. 2010) supports the hypothesis that they may be a more favourable source of trace elements (Kadar et al. 2012) than the bulk analogues to improve the life functions of various organisms. Hong et al. (2005) demonstrated that TiO_2 nanoparticles (n-TiO_2) markedly increased the growth of spinach seeds by stimulating photosynthesis. Mahajan et al. (2011) described the positive effect of ZnO nanoparticles on the growth of *Cicer arietinum*

and *Vigna radiata*. Zinc is a nutrient essential for plants; while bulk ZnO is very insoluble in water, its nanoscale counterpart was observed to be more bioavailable and highly suitable for seed germination.

The zero-valent iron nanoparticles (nZVI) are primarily known as a remediation agent for in situ treating contaminated aquifers and soils. Relatively low standard reduction potential (E_H^o (Fe^{2+}/Fe^0)=−0.44 V) allows nZVI to act as electron donors to catalyse a wide range of different reactions (Li et al. 2006). Application of nZVI in green technologies can have both positive and negative effects. The negative effect is associated with considerable nZVI toxicity related to its redox activity (Ševců et al. 2011). The complete mechanism of nZVI action has not yet been fully elucidated. Briefly, oxidation of nZVI leads to the formation of reactive oxygen species (ROS) via the Fenton reaction, which gives rise to oxidative stress in treated cells. Reactions of ROS with biological macromolecules result in lipid peroxidation, protein oxidation and DNA damage (Ševců et al. 2011). On the other hand, application of nZVI to contaminated sites can generally reduce ecotoxicity of the environment due to the negligible effect of nZVI compared to the toxicity caused by the respective pollution (Nemecek et al. 2014).

Iron is known to be an essential microelement in a number of processes occurring in prokaryotic and eukaryotic organisms including respiration, photosynthesis, oxygen transport or cell proliferation. However, for the organisms to acquire iron from the environment is difficult because it is found mainly in insoluble Fe^{3+} form. The bioavailability of iron is highly dependent on the capabilities of the organism and on the chemical origin of the iron. Cells have developed a complex of mechanisms for Fe management that coordinates iron uptake, further utilization and storage to maintain the vitally important iron homeostasis (Bleackley and MacGillivray 2011). However, these mechanisms usually enable uptake only of ferrous ions, and thus, extracellular reduction of ferric iron is the limiting step in iron uptake (Andrews et al. 1999). In contrast, better bioavailability of nZVI supports its ready interaction with the cell surface, which facilitates nZVI penetration into cytosol. Moreover, nZVI can be internalized by endocytosis (Ševců et al. 2011). In addition, nZVI oxidation to ferrous ions (suitable form for iron uptake) (Adeleye et al. 2013) could also contribute to improving the bioavailability of iron.

The effect of optimum iron concentration on microalgae growth was reported by Huang et al. (2014). Kadar et al. (2012) compared commonly used Fe-EDTA with three nZVI forms with distinct surface properties. At the concentrations used, nZVI did not cause any harmful effect and in fact it led to a better growth of the algae.

Photosynthetic microorganisms have received widespread attention in association with lipid accumulation and biodiesel production (Griffiths and Harrison 2009). The economic feasibility of mass culture greatly depends on high biomass productivity and yield of valuable lipid. Therefore, extensive studies are required to find optimum culture conditions. An effect of nZVI-amended media on the growth and physiological properties of several oleaginous microalgae and one cyanobacterium was investigated, with the focus on the effects of stimulatory nZVI concentrations. Further, an overall lipid and fatty acid composition in cells as dependent on nZVI concentration was examined.

Experiments and methods

Zero-valent iron nanoparticles Dispersions of two different types of nZVI, Nanofer 25 and Nanofer 25S, were purchased from Nanoiron Ltd., Rajhrad, Czech Republic. Nanofer 25 is a pure water suspension of nanoparticles with no additives. Nanofer 25S, a stabilized form of the Nanofer 25 product, is comprised of nZVI surface-coated with a Na-acrylic copolymer.

Cultivation of algae and cyanobacterium The green algae *Desmodesmus subspicatus* (strain Brinkmann 1953, CCALA 688), *Dunaliella salina* (strain Massjuk 1962/9, CCALA 340), *Parachlorella kessleri* (strain LARG 1, CCALA 253) and *Raphidocelis subcapitata* (strain Skulberg 1959/1, CCALA 433); the eustigmatophycean algae *Nannochloropsis limnetica* (strain Krienitz 1998/3, SAG 18.99) and *Trachydiscus minutus* (strain Lukavský et Přibyl 2005/1, CCALA 931); and the cyanobacterium *Arthrospira maxima* (strain Compere 1968/3786, CCALA 027) were obtained from the Culture Collection of Autotrophic Organisms at the Institute of Botany, ASCR (Třeboň, Czech Republic), and from the SAG Culture Collection of Algae (Göttingen, Germany).

The screening of optimum concentrations of nZVI was performed for five strains in 96-well microplates (supplementary Fig. 1S) (Lukavský et al. 2003). The plates were filled with Zehnder medium Z8 (Staub 1961) with Fe-EDTA as iron source (0.5 mg L^{-1} Fe) as control and Zehnder medium Z8 that was spiked by different concentrations of Nanofer 25 and Nanofer 25S. Microplates (96 well) were sterilized by UV radiation (20 min), then inoculated to a final concentration of 100,000 cells mL^{-1}, sealed with polyethylene foil and lid and cultured in a cultivation unit at 30 °C and light intensity of 10 W m^{-2} (from fluorescent tubes, day light). CO_2 was supplied in 2 % (vv^{-1}). Growth was evaluated as optical density at 750 nm (with iEMS reader, Labsystems, Helsinki, Finland) at 72-h intervals.

The estimation of biomass yield and total lipid content was performed in 1-L bottles containing either Nanofer-25-free Zehnder medium Z8 as a control or the same medium with different concentrations of Nanofer 25. Irradiation was

provided from one side by fluorescent tubes (day light) at 10 W m^{-2} and aeration by bubbling with air enriched with 2 % CO_2 at 27 °C (supplementary Fig. 2S). The cells were harvested after 216 h by centrifugation and stored lyophilized until analysis.

The cultivation conditions of inocula used in all experiments were the same as in the screening experiment, i.e. Zehnder medium Z8, 30 °C and light intensity 10 W m^{-2} (from fluorescent tubes, day light). The inocula were taken from exponential phase cultures.

Analysis of lipids and fatty acids Acetonitrile, 2-propanol, hexane, chloroform and methanol were purchased from Sigma-Aldrich (Prague, CR). The extraction procedure was based on the method of Bligh and Dyer (1959), except that 2-propanol was substituted for methanol since isopropanol does not serve as a substrate for phospholipases (Kates 1986). The alcohol-water mixture of cells was cooled, one part of chloroform was added and the lipids were extracted for 30 min. Insoluble material was sedimented by centrifugation, and the supernatant was separated into two phases. The aqueous phase was aspirated off, and the chloroform phase was washed three times with two parts 1 M KCl each. The resulting chloroform phase was evaporated to dryness under reduced pressure.

The total TAGs (~5 mg) were saponified overnight in 10 % KOH-MeOH at room temperature. The fatty acid fraction obtained from the saponification was partitioned between alkali aqueous solution (pH 9) and diethyl-ether to remove basic and neutral components. The aqueous phase containing fatty acids was acidified to pH 2 and extracted with hexane. The fatty acid fraction was methylated using BF_3/MeOH (14 % solution of BF_3 from Sigma-Aldrich).

Gas chromatography-mass spectrometry (GC-MS) of fatty acid methyl ester (FAME) was done on a GC-MS system consisting of Varian 450-GC (Varian BV), Varian 240-MS ion trap detector with electron ionization (EI) and CombiPal autosampler (CTC, USA) equipped with split/splitless injector. A DB-5MS column was used for separation (60 m, 0.25 mm ID, 0.25 μm film thickness). The temperature program started at 60 °C and was held for 1 min in splitless mode. Then the splitter was opened and the oven was heated to 160 °C at a rate of 25 °C min^{-1}. The second temperature ramp was up to 280 °C at a rate of 2.5 °C min^{-1}, this temperature being maintained for 10 min. The solvent delay time was set to 8 min. The transfer line temperature was set to 280 °C. Mass spectra were recorded at 3 scans s^{-1} under electron ionization at 70 eV, mass range *m/z* 50–450. FAMEs were identified according to their mass spectra (1992; Dembitsky et al. 1991) and using a mixture of chemical standards obtained from Sigma-Aldrich.

Statistical analysis To perform multivariate statistical analyses, we used the program CANOCO 5 (Microcomputer Power, USA). Since the gradient lengths in the data determined by detrended correspondence analysis were short, we used a linear method (principal component analysis, PCA) to visualize the major variances within the dataset (Šmilauer and Lepš 2014). A direct method (redundancy analysis, RDA) was then used to analyse the significance of the nZVI effects on fatty acid composition. To eliminate the effect of taxonomy, the species were transformed into dummy variables and set as covariates. The effect of nZVI was tested using the Monte Carlo permutation test; permutation blocks were defined by covariates (Šmilauer and Lepš 2014).

Results

Screening experiments—effect of nanoparticles on cell growth

Several algae and cyanobacterium were cultivated in an exponentially increasing range of nZVI concentrations in order to evaluate the effect of different nZVI dilutions on their growth. In microplate cultivations, the growth dependencies of all treated microorganisms on nanoparticle concentrations showed a similar character. Figures 1 and 2 illustrate the results obtained with the microalga *P. kessleri* and the cyanobacterium *A. maxima*. Enhancement of growth was observed at concentrations ranging from 0.5 to 5.1 mg L^{-1} whereas above 17 mg L^{-1} the growth was gradually reduced. A similar effect was observed in other treated microorganisms (Table 1).

The growth inhibition of Nanofer 25 (Figs. 1a and 2a) during the 216 hours of cultivation was significantly lower than that of Nanofer 25S (Figs. 1b and 2b). On the other hand, the stimulatory effect of Nanofer 25S was less pronounced compared to Nanofer 25. This difference could be demonstrated on *P. kessleri* (Fig. 1). At the end of the experiment, the biomass obtained in cultivation with Nanofer 25 (5.1 mg L^{-1}) was 25 % higher in comparison with the same concentration of Nanofer 25S. In *A. maxima* (Fig. 2), the biomass yield with Nanofer 25 (5.1 mg L^{-1}) was almost 9 % higher compared to Nanofer 25S (5.1 mg L^{-1}). Based on this result, Nanofer 25 was chosen for further batch experiments.

Biomass yield and lipid content of nZVI-treated microorganisms

To confirm growth stimulation, a series of batch cultivations were carried out at two growth-stimulating concentrations of Nanofer 25 (1.7 and 5.1 mg L^{-1}). In addition, the content of lipids and fatty acid composition under the two sets of conditions were analysed.

The biomass yield and total lipid content were determined in all strains under study (Table 2). In comparison with the

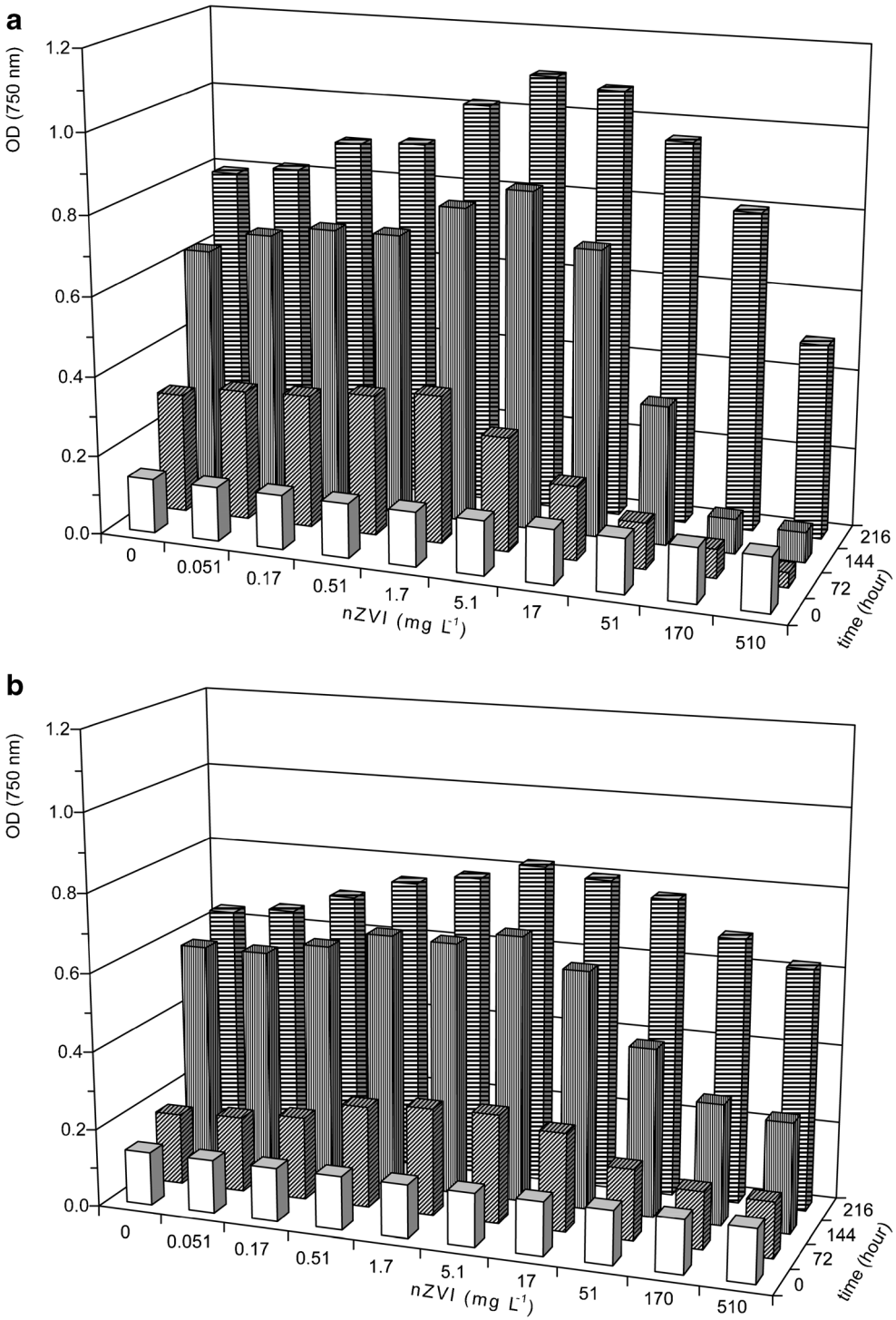

Fig. 1 Effect of different concentrations of Nanofer 25 (**a**) and Nanofer 25S (**b**) nanoparticles on the growth of *Parachlorella kessleri*. Optical density (*OD*)

control medium, enhancement of cell growth was observed. Furthermore, stimulation of lipid syntheses was observed as a result of stress conditions. The highest values of both parameters (lipid and biomass accumulation) were obtained in cultivation at 5.1 mg L^{-1} nZVI. Both strains of *P. kessleri* and *T. minutus* exhibited fairly high biomass yield and lipid production. Untreated population of *P. kessleri* reached maximal biomass accumulation of 19.4 g L^{-1} and lipid content of 27.1 %. The exposure to nZVI (5.1 mg L^{-1}) led to an improvement of both biomass yield (29.3 g L^{-1}) and lipid content (30.9 %).

T. minutus (supplementary Fig. 3) also exhibited a growth and lipid content enhancement as a result of nZVI (5.1 mg L^{-1}) treatment. The biomass (10.4 g L^{-1}) was increased to 13.7 g L^{-1} and lipid content from 26.2 to 35.0 %. A significant increase in lipid content in relation to total dry

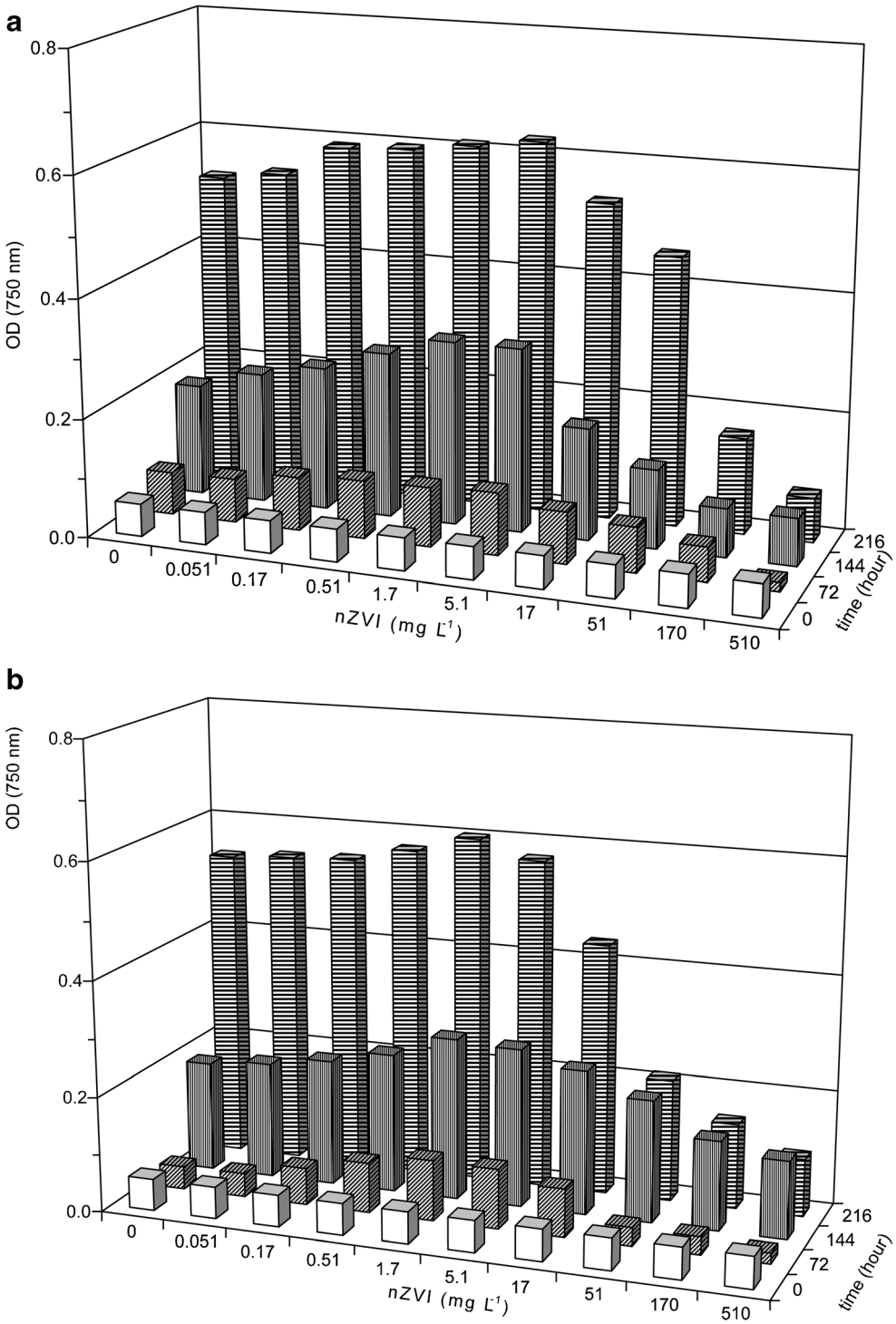

Fig. 2 Effect of different concentrations of Nanofer 25 (**a**) and Nanofer 25S (**b**) nanoparticles on the growth of *Arthrospira maxima*. Optical density (*OD*)

Table 1 Growth stimulation (% increase in OD) at different Nanofer 25 concentrations (after 216 h of cultivation)

Species/mg L^{-1} Nanofer 25	0	0.051	0.17	0.51	1.7	5.1	17	51	170	510
Arthrospira maxima	951	976	1073	1084	1109	1136	967	825	296	136
Desmodesmus subspicatus	414	426	438	523	567	718	358	96	29	32
Parachlorella kessleri	579	595	653	660	740	800	782	700	584	357
Raphidocelis subcapitata	375	372	375	387	453	484	414	258	161	141
Trachydiscus minutus	776	886	914	914	938	908	757	621	514	493

Table 2 Fatty acid profile (% ww^{-1}), dry weight and total lipids of seven cyanobacterial and microalgal strains cultivated at different Nanofer 25 concentrations

Species	14:0	16:0	16:1	16:2	16:3	16:4	18:0	18:1w9	18:2w6	18:3w6	18:3w3	18:w4	20:4w6	20:5w3	DW (g L^{-1})	Total lipids (g L^{-1})	Percent
Arthrospira maxima control	2.4	35.9	4.5	0.0	0.0	0.0	5.5	6.1	28.0	0.0	17.6	0.0	0.0	0.0	2.0	0.35	17.6
A. maxima 1.7[a]	1.9	32.2	6.1	0.0	0.0	0.0	4.1	4.2	33.4	0.0	18.1	0.0	0.0	0.0	2.3	0.43	18.2
A. maxima 5.1[a]	0.8	29.3	7.5	0.0	0.0	0.0	3.2	0.2	37.8	0.0	21.2	0.0	0.0	0.0	2.3	0.49	21.4
Dunaliella salina control	0.2	25.2	3.5	0.0	0.0	18.1	3.5	16.2	9.6	1.3	22.4	0.0	0.0	0.0	3.5	0.84	24.0
D. salina 1.7	0.1	20.7	3.9	0.0	0.0	21.8	2.6	13.2	11.7	1.3	24.7	0.0	0.0	0.0	4.8	1.34	27.9
D. salina 5.1	0.1	16.7	4.1	0.0	0.0	25.0	1.2	8.3	13.8	1.4	29.4	0.0	0.0	0.0	5.4	1.73	32.0
Desmodesmus subspicatus control	0.7	23.9	6.1	1.0	2.1	4.8	5.7	19.8	19.4	1.9	14.6	0.0	0.0	0.0	1.5	0.36	24.0
D. subspicatus 1.7	0.6	20.0	5.2	1.3	2.6	5.3	4.5	16.4	24.5	2.5	17.1	0.0	0.0	0.0	1.9	0.63	33.0
D. subspicatus 5.1	0.4	10.4	4.5	1.9	3.4	6.9	3.3	8.5	34.8	3.2	22.7	0.0	0.0	0.0	2.6	0.99	38.0
Nannochloropsis limnetica control	3.0	21.4	30.4	0.0	0.0	0.6	4.3	11.7	3.7	0.3	0.5	0.0	4.2	19.9	6.2	2.17	35.0
N. limnetica 1.7	2.2	16.4	25.6	0.0	0.0	0.8	3.2	8.7	12.8	0.4	0.8	0.0	5.3	23.8	7.1	2.91	41.0
N. limnetica 5.1	1.4	11.8	21.7	0.0	0.0	1.3	2.4	6.5	14.9	0.5	1.1	0.0	6.9	31.5	7.4	3.55	48.0
Parachlorella kessleri control	2.2	34.3	3.3	5.5	6.4	0.0	18.1	16.6	7.1	0.1	6.4	0.0	0.0	0.0	19.4	5.26	27.1
P. kessleri 1,7	2.0	32.2	3.0	5.9	6.7	0.0	17.3	14.5	10.5	0.1	7.8	0.0	0.0	0.0	23.1	6.77	29.3
P. kessleri 5,1	1.0	20.5	1.3	6.5	7.1	0.0	14.2	13.7	21.4	0.1	14.2	0.0	0.0	0.0	27.5	8.72	31.7
Raphidocelis subcapitata control	0.7	23.0	3.5	0.0	0.0	5.3	13.9	23.5	6.7	0.0	14.1	9.3	0.0	0.0	1.2	0.05	4.2
R. subcapitata 1.7	0.6	12.6	3.5	0.0	0.0	6.9	13.0	17.8	7.1	0.0	24.7	13.8	0.0	0.0	1.7	0.09	5.3
R. subcapitata 5.1	0.5	6.8	3.2	0.0	0.0	7.8	8.4	13.4	9.2	0.0	31.2	19.5	0.0	0.0	2.3	0.14	6.1
Trachydiscus minutus control	22.8	22.4	6.9	0.0	0.0	0.0	3.6	1.8	4.4	0.0	0.0	0.0	0.1	38.0	10.4	2.72	26.2
T. minutus 1.7	22.5	21.7	4.9	0.0	0.0	0.0	1.4	1.3	5.6	0.0	0.0	0.0	0.2	42.4	12.1	3.61	29.8
T. minutus 5.1	20.0	13.2	3.8	0.0	0.0	0.0	1.1	0.9	9.4	0.0	0.0	0.0	0.7	50.9	13.7	4.78	34.9

Standard deviations of all relative percent concentrations in this table are <0.13 as calculated from three replicates

[a] Concentration of Nanofer 25 (mg L^{-1})

biomass has been observed in the algae *N. limnetica*, *D. subspicatus*, *D. salina* and *R. subcapitata*. However, no significant biomass yield was detected in these strains. The cultivation of *N. limnetica* in a medium with 5.1 mg L^{-1} nZVI led to lipid accumulation of 48 %, but biomass production was rather low (7.4 g L^{-1}). A comparable phenomenon was observed in *D. subspicatus*. Cultivation on a medium with 5.1 mg L^{-1} nZVI led to lipid accumulation as high as 38 % while the biomass reached a mere 2.6 g L^{-1}. Similarly, in *D. salina*, the total lipid content under nZVI exposure was established at 32 % but the biomass yield was only 5.4 g L^{-1}. At the optimal concentration of nZVI, the strain of the green alga *Raphidocelis* produced 2.3 g L^{-1} of dry biomass and the amount of total lipids was increased by almost half compared to the control. In terms of potential biotechnological use, it is thus not easy to find a suitable strain with optimal properties that determine the cost of the target product.

The cyanobacterium *A. maxima* was chosen as a model prokaryotic photosynthetic organism. The response to nZVI treatment was different and not so significant compared to the studied microalgal strains. Cultivation on nZVI-amended medium influenced only lipid accumulation. The maximum was reached at 5.1 mg L^{-1} nZVI, i.e. 21.4 % (see Table 2).

Fatty acid composition of nZVI-treated microorganisms

The fatty acid (FA) composition of the cultivated strains was very diverse and dependent on strain taxonomy and concentration of nZVI (Table 2). PCA identified the taxonomic identity of the studied strains as the major source of variability within the dataset. The variability explained by the first principal axis (54.3 %) can be attributed to the differences between *T. minutus* and *N. limnetica* (with high proportion of E and A represented) and the other strains (with E and A completely lacking, Fig. 3).

Analyses showed that cultivation on nZVI-amended medium brought about significant changes of FA profiles. The effect of cultivation conditions (nZVI concentration) on single FA production was analysed using RDA (graph not shown). When the effect of taxonomy on FA composition was filtered out using covariate, nZVI concentration explained 62.9 % of the remaining variability and its effect was statistically significant (p=0.001).

The general response of treated microorganisms to different nZVI concentrations was similar. In all strains, the abundance of polyunsaturated FAs (PUFAs) increased with nZVI concentration. The most significant increase in the proportion of PUFAs was observed in the microalga with 50.9 % of eicosapentaenoic acid in the cultivation containing 5.1 mg L^{-1} nZVI (Table 2 and Fig. 3).

In contrast to the increasing abundance of PUFAs, the content of saturated and monounsaturated FAs decreased, with the exception of monounsaturated palmitoleic acid (16:1, Po). The

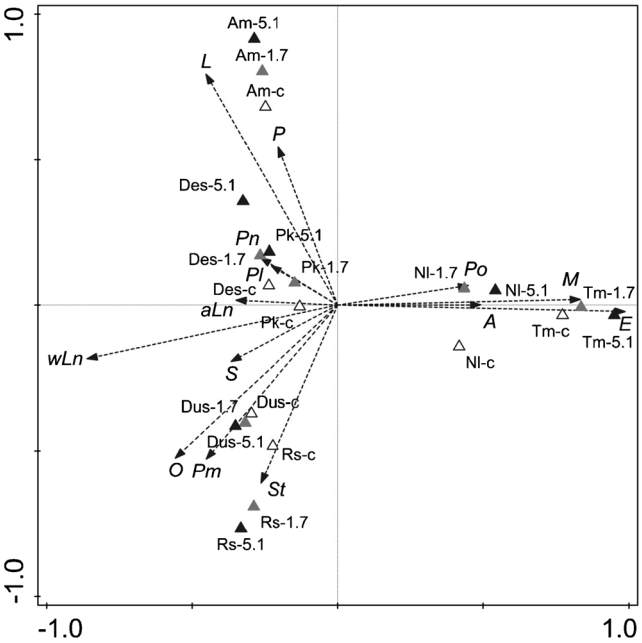

Fig. 3 Principal component analysis (PCA) of the relative proportions of FAs in treated microalgae and cyanobacterium strains cultivated in Zehnder Z8 medium (control) and Nanofer 25 (1.7 mg L^{-1}, 5.1 mg L^{-1}) Z8 modified medium. The first two axes are displayed; they explained 69.7 % of the variability. Abbreviations of fatty acids and microorganisms (the abbreviation is in brackets): (i) Fatty acids: 14:0 (M), 16:0 (P), 16:1 (Po), 16:2 (Pl), 16:3 (Pn), 16:4 (Pm), 18:0 (S), 18:1w9 (O), 18:2w6 (L), 18:3w6 (aLn), 18:3w3 (wLn), 18:4w3 (St), 20:4w6 (A), 20:5w3 (E); Microorganisms: *A. maxima* (Am). *D. salina* (Dus), *D. subspicatus* (Des), *N. limnetica* (Nl), *P. kessleri* (Pk), *R. subcapitata* (Rs), *T. minutus* (Tm)

abundance of the palmitoleic acid in the cyanobacterium *A. maxima* and microalga *D. salina* increased simultaneously with PUFAs. A different response of palmitoleic acid was observed in the microalga *R. subcapitata*, its abundance being almost constant throughout the tested range of nZVI concentrations (Table 2).

Discussion

Biomass yield and lipid content of nZVI-treated microalgae

Our experiments showed a stimulation of cell growth and lipid production in the studied microorganisms by nZVI. In general, the highest lipid and biomass accumulation was obtained in cultivations at 5.1 mg L^{-1} of nZVI. nZVI oxidation to other iron species decreased the concentration of nZVI during the experiment (Adeleye et al. 2013). Nevertheless, the augmentation of microalgae growth supports the hypothesis that nanoparticles represent a suitable source of trace elements (Kadar et al. 2012). Since stress conditions (nutrient or another stress) induce a higher lipid production in oleaginous microorganisms (Kang et al. 2014), enhanced lipid accumulation in the presence of nZVI can be connected with oxidative stress caused by the exposure of the cells to nZVI particles (Ševců

et al. 2011). Like nZVI, TiO_2 nanoparticles have the ability to induce generation of ROS (Kang et al. 2014). In their study, Kang et al. (2014) proved the generation of ROS and higher lipid accumulation after exposure of *Chlorella vulgaris* UTEX 265 to 0.1 g L^{-1} TiO_2 nanoparticles.

Control cultivation of *P. kessleri* versus cultivation with nZVI at the concentration at 5.1 mg L^{-1} nZVI led to improvement of both biomass and lipid content from 19.4 to 29.3 g L^{-1} and from 27.1 to 30.9 %, respectively. The highest lipid content (51 %) was reported by Pribyl et al. (2012) in the strain *P. kessleri* CCALA 255 while biomass yield (6.5 g L^{-1}) was significantly lower than that found in the present study.

The biomass and lipid content of *T. minutus* increased from 10.4 to 13.7 g L^{-1} and lipid content from 26.2 to 35 % when this alga was cultivated with an nZVI concentration of 5.1 mg L^{-1} (Table 2). Similar values were obtained by Gigova et al. (2012) who studied the effect of temperature and light intensity on the same *T. minutus* strain. They reported that low temperature (about 15 °C), which represents a stress condition for this microorganism, increased lipid accumulation to 35 %. On the other hand, pronounced growth inhibition was found under these conditions (Gigova et al. 2012).

No significant biomass yield was detected in the other strains of algae, i.e. *N. limnetica*, *D. subspicatus*, *D. salina* and *R. subcapitata*. This is very common, since many microalgae strains can accumulate lipids in large amounts but show very slow growth or low biomass accumulation due to small cell size (Griffiths and Harrison 2009). *N. limnetica* is able to accumulate lipids in the range from 31 to 68 %, depending on the strain and culture conditions (Krongkan et al. 2013).

The production of lipids in cultivation of *D. subspicatus* at a concentration of 5.1 mg L^{-1} nZVI reached about 38 % of biomass. These results correspond with those reported by Abd El Baky et al. (2012) who studied an effect of Fe^{3+} concentration and CO_2 level in another member of the Scenedesmaceae, *Scenedesmus obliquus*. Maximum *S. obliquus* biomass yield (2.4 g L^{-1}) was found at 10 mg L^{-1} Fe^{3+} and 3 % CO_2 whereas the highest lipid accumulation of 34 % was reached at 15 % CO_2 and 2.5 mg L^{-1} Fe^{3+}.

The results obtained with the cyanobacterium *A. maxima* after treatment with nZVI were not significant; the amount of lipids increased while biomass did not.

Fatty acid composition of nZVI-treated microorganisms GC-MS was used for the qualitative and quantitative analysis of fatty acids as methyl esters. The data showed that production of PUFAs increased with nZVI concentration. On the other hand, the content of saturated and monounsaturated FAs decreased, with the exception of palmitoleic acid (Table 2 and Fig. 3).

The increase of PUFAs was also noticed by Kang et al. (2014) who investigated the relationship between intracellular lipid production and TiO_2 nanoparticle-induced oxidative stress in *C. vulgaris* UTEX 265. The possible explanation of this phenomenon could involve cellular response to nanoparticle-induced oxidative stress. Under oxidative stress, numerous radicals affect various physiological functions. Thanks to two or more double bonds in the molecule, PUFAs have a radical scavenging potential which contributes to cell protection against increased ROS level (Adarme-Vega et al. 2014). This suggests that high accumulation of unsaturated fatty acids may play an important role in protection mechanisms.

The studied strain of *T. minutus* is known for a high production of eicosapentaenoic acid (20:5w3) (Rezanka et al. 2011), which belongs to the group of PUFAs with long chains that are pharmacologically very important and are effective in preventing or treating some diseases (Mata et al. 2010). The maximum production was found to reach 39 % of total FAs under phosphorus starvation (Rezanka et al. 2011). In the present study, cultivation of the same *T. minutus* strain with nZVI (5.1 mg L^{-1}) showed a considerable increase in the eicosapentaenoic acid proportion to 51 % (Table 2) of total FAs. Combined with the positive effect of nZVI on total lipid content, the addition of this Fe source may represent an effective way how to increase the production of this fatty acid.

In conclusion, a stimulatory effect of nZVI on the biomass production and lipid content of several microalgal and cyanobacterial strains was determined. The changes in lipid metabolism could be associated with oxidative action of nZVI. The maximal biomass yield of microalgae was observed at 5.1 mg L^{-1} Nanofer 25 and at 1.7–5.1 mg L^{-1} in the cyanobacterium; in addition, the concentration of 5.1 mg L^{-1} significantly enhanced lipid accumulation. Moreover, treatment with nZVI elicited changes in FA's composition and caused a higher proportion of polyunsaturated FAs. Further research in this field could contribute to increasing the nutritive value of produced lipids and acquiring economically favourable products.

Acknowledgments The research was supported by GACR P503/11/0215 and GACR 14-00227S, by Competence Centres TE01020218 and Biorefinery Res. Centre of Competence TE 01020080 grants of the Czech Technology Agency, by the Institutional Internal Project RVO61388971, by ICT IGA project no. A2 FPBT 2014 022, and Centre for Algal Biotechnologies (Algatech) project, reg. no. CZ.1.05/2.1.00/03.0110

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