Folia Microbiol (2016) 61:329–335
DOI 10.1007/s12223-015-0442-7

Enhancing the lipid productivity of yeasts with trace concentrations of iron nanoparticles

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Received: 17 September 2015 / Accepted: 10 December 2015 / Published online: 18 December 2015

Abstract Oxidative stress induced by zero-valent iron nanoparticles (nZVIs) was used to improve lipid accumulation in various oleaginous and non-oleginous yeasts—*Candida* sp., *Kluyveromyces polysporus*, *Rhodotorula glutinis*, *Saccharomyces cerevisiae*, *Torulospora delbrueckii*, *Trichosporon cutaneum*, and *Yarrowia lipolytica*. The highest lipid yields occurred at 9–13 mg/L nZVIs. Gas chromatography-mass spectrometry was used for the quantitative and qualitative analysis of the fatty acids. It showed an increasing abundance of polyunsaturated fatty acids, especially essential linoleic acid, in the presence of nZVIs. Our results suggest that nZVIs can be used to improve not only lipid production by oleaginous microorganisms but also the nutritional value of biosynthesized unsaturated fatty acids.

Introduction

Current microbes essentially "remember" the time they lived in a reducing environment (more than 2 billion years ago). The oxygen content in the atmosphere was then much lower than today, and the environment contained compounds of divalent iron (Kasting 1993). Modern microorganisms substantially maintain this requirement for this form of the transition metal. Iron in the form of compounds is often used as enzyme cofactors in many biochemical processes, and is usually in two valence states, in which it receives or loses an electron, and is therefore used in enzymatic reactions for the transfer of electrons. Unfortunately, in the presence of atmospheric oxygen, iron is oxidized, and as ferric ion, it forms oxyhydroxide colloids, whose water solubility in a neutral environment is about 10^{-9} mol/L. This need not cover the needs of many microorganisms. In culture, this drawback can be removed by either acidifying the culture medium, wherein at pH 4, the solubility of these compounds increases by about 3 orders of magnitude (Chipperfield and Ratledge 2000), by reducing the ferric to ferrous ions or by using chelating agents produced by the microorganisms (siderophores) or added to culture medium, e.g., ethylenediaminetetraacetic acid (EDTA). In our experiment, we used another method, i.e., the use of zero-valent iron nanoparticles (nZVI) as an iron source. Except for the effects of oxidative stress, nZVI is also gradually oxidized from the zero-valent Fe over ferrous to ferric ions (Zhang 2003) and the continuous supply of ferrous ions can cause the biochemical changes described below.

Zero-valent iron nanoparticles have the ability to induce oxidative stress (Ševců et al. 2011). The nZVI is oxidized through the mechanism of Fenton reaction to generate reactive oxygen species (ROS), including hydroxyls, superoxide radical, and hydrogen peroxide. These oxygen forms damage biological molecules, and their products can be lethal (Keenan et al. 2009). On the other hand, microorganisms possess both enzymatic and non-enzymatic defense mechanisms, which protect them against the action of ROS to a certain extent (Jamieson 1998).

Electronic supplementary material The online version of this article (doi:10.1007/s12223-015-0442-7) contains supplementary material, which is available to authorized users.

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The connection between ROS production and higher lipid accumulation was proved by Kang et al. (2014). They applied TiO_2 nanoparticles as a source of oxidative stress in oleaginous green alga *Chlorella vulgaris*. The same effect of ROS on lipid accumulation was observed in PC12 cancer cells as a response to the action of CdTe nanoparticles. An experiment with PC12 cells pre-treated with the antioxidant molecule *N*-acetylcysteine proved the connection between enhanced lipid accumulation and ROS generation as a response to nanoparticle action (Khatchadourian and Maysinger 2009).

Apart from generation of oxidative stress, nZVI can influence the function of some proteins. Fajardo et al. (2013) described an up-regulation of the enzymes alanine dehydrogenase and glutamate dehydrogenase, enzymes involved in tricarboxylic acid cycle (TCA). Their up-regulation would lead to a stimulation of TCA cycle and further increase in energy generation (Fajardo et al. 2013). In this context, additional energy from TCA cycle could entail enhancement of biosynthesis of storage lipids.

Oleaginous yeast can accumulate lipids to levels greater than 20 % (Meng et al. 2009) or even 26 % in Santamauro et al. (2014) of their dry mass. The lipids are accumulated in the form of lipid droplets which serve mainly as energy deposits (Ageitos et al. 2011), and their synthesis is enhanced mainly under stress conditions (Rodolfi et al. 2009). Therefore, current studies are also focused on the development of stress conditions (e.g., nitrogen or phosphate starvation) aimed at improving lipid accumulation. On the other hand, nutrient starvation can lead to inhibition of cell division (Widjaja et al. 2009). The effect of nZVIs was studied in several microalgae strains (Kadar et al. 2012; Pádrová et al. 2015). In these studies, trace concentrations of nZVIs not only enhanced the lipid accumulation but also stimulated the growth of all algal strains.

In the present study, we report the first investigation focused on the application of nZVI as an agent stimulating the lipid production by seven yeast strains (*Candida* sp., *Kluyveromyces polysporus*, *Rhodotorula glutinis*, *Saccharomyces cerevisiae*, *Torulospora delbrueckii*, *Trichosporon cutaneum*, *Yarrowia lipolytica*). In addition, we showed a dependence of the yeast growth, lipid production, and fatty acid profiles on nZVI concentration.

Experimental

Materials

Dispersion of zero-valent iron nanoparticles, Nanofer 25, was purchased from Nanoiron Ltd. (Rajhrad, Czech Republic). Nanofer 25 is an aqueous suspension of uncoated iron nanoparticles, stabilized by an inorganic modifier. Nanofer 25 is characterized by manufacturer's information: mean particle size, 50 nm; specific surface area, >25 m^2/g; and purity of iron in solid phase, 85 %.

Microorganisms

The yeast strains used in the present study were *Candida* sp. DBM 2163; *Kluyveromyces polysporus* DBM 2171 (CCY 30-5-10); *Rhodotorula glutinis* CCY 20-2-20; *Saccharomyces cerevisiae* DBM 2115; *Torulaspora delbrueckii* DBM 39; *Trichosporon cutaneum* CCY 30-5-10; and *Yarrowia lipolytica* CCY 29-26-36 supplied by Culture Collection of Yeast, Institute of Chemistry, Slovak Academy of Sciences, Bratislava and by Collection of Yeasts and Industrial Microorganisms of the University of Chemistry and Technology, Prague. Stock cultures were maintained in 20 % of glycerol at −60 °C. Malt extract agar (23 g/L, pH 7) was employed for short-term storage.

Cultivation conditions

nZVI was not dispersed; we used fresh suspension of nanoparticles from the supplier, who guaranteed a 2-month stability of the stock suspension.

Growth screening experiments were performed in the above yeast strains cultivated with concentrations of nZVI (mg/L): 1.7, 5.1, 9, 13, 17, 51, 170, and 510. The experiments were performed in the Bioscreen C (Labsystems, Finland) device in microtiter plates, see, e.g., Figs. 1S-3S (Supplements). Cultivation temperature was 30 °C; absorbance (A) was determined every 2 h for 72 h. All experiments were performed five times. To determine lipid content and biomass yield, the yeasts were cultivated in 250 mL mineral medium in Erlenmeyer flasks (6 mL inoculum) on an orbital rotary shaker (150 rpm) at 30 °C in triplicate parallels. The inoculation was at biomass absorbance (A_{440}) = 0.2. Mineral medium composition for control was as follows (g/L): $(NH_4)_2SO_4$, 5; YNB (yeast nitrogen base w/o amino acids and ammonium sulfate), 1.7; and glucose, 10. For examining the effect of nZVI, the mineral medium was amended by 5, 9, and 13 mg/L nZVI. The cultivations were carried out for 72 h (until early stationary phase). Reduction of nZVI content during cultivation has not been determined. After the cultivation, the cells were centrifuged (9000*g*, 10 min) and washed two times. Biomass was frozen at −75 °C and lyophilized.

Analysis of lipids and fatty acids

The extraction and isolation of total lipids from yeast suspension was performed according to the methodology previously described (Řezanka et al. 2013). Briefly, the yeast suspension was ground with glass beads under liquid nitrogen and the crushed yeast was extracted according to Bligh and Dyer (1959).

Fatty acid methyl esters (FAMEs) were prepared after saponification and further esterification using BF_3/MeOH (Řezanka 1993). The products were dissolved in hexane and analyzed by gas chromatography-mass spectrometry on a Varian GC-MS system with the split/splitless injector (250 °C) and a SP™-2380 Capillary GC Column L × I.D. 60 m × 0.25 mm, *df* 0.20 μm. Helium was used as carrier gas at 1.0 mL/min. The split/splitless injection port was maintained at 255 °C. The split ratio was 1:90, and the injection volume was 1 μL. The temperature program was as follows: 150 °C for 1 min, subsequently increasing at 20 °C/min to 180 °C and at 2 °C/min to 250 °C, which was maintained for 1 min. FAMEs were identified according to their mass spectra (Vančura et al. 1987; Dembitsky et al. 1993; Dembitsky and Řezanka 2005) and using a mixture of chemical standards obtained from Sigma-Aldrich. All experiments concerning the analysis of FAMEs and their derivatives were carried out by electron impact MS.

Lipid peroxidation assay

The extent of lipid peroxidation can be expressed as thiobarbituric acid-reacting substances (TBARS). The yeast was cultivated in mineral medium and mineral medium amended with nZVI (the highest monitored concentration 13 mg/L). During the cultivation with nZVI, the level of TBARS was measured in 24-h intervals. Cells were separated by centrifugation (9000*g*, 10 min) and resuspended in 50 mmol/L sodium phosphate buffer (pH 7). Before the analyses, the cells were disrupted by a mixer mill (MM 400, Retsch, Germany) using a steel ball (12 mm). The cell disintegrate (1 mL) was mixed with 1 mL of thiobarbituric acid solution (29 mmol/L 2-thiobarbituric acid in 8.75 mol/L acetic acid). The mixture was heated at 95 °C for 1 h and cooled on ice, then 3.5 mL of *n*-butanol was added and vortexed. Organic layer was separated by centrifugation (9000*g*, 10 min), and its absorbance was measured at 532 nm. 1,1,3,3-Tetraethoxypropane was used as a standard. The level of lipid peroxidation was determined in 24-h intervals during 4-day cultivation.

Statistical analysis

Multivariate statistical analyses were performed by using 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 variability of major variances within the dataset (Šmilauer and Lepš 2003). A direct method (redundancy analysis, RDA) was then applied to analyze the significance of the nZVI effects on fatty acid composition. The effect of taxonomy was eliminated by transforming the species into dummy variables and setting them as covariates. The effect of nZVI was tested using Monte Carlo permutation test; permutation blocks were defined by covariates (Šmilauer and Lepš 2003).

Results and discussion

Analysis of fatty acid

Analysis of fatty acid composition (see the percentages of saturated, monounsaturated, and polyunsaturated fatty acids in Tables 1 and 2) showed that increased concentration of added nZVI significantly decreases the amount of saturated fatty acids (FAs): predominantly even-chain acids, i.e., palmitic (16:0), stearic (18:0), and arachidic acids (20:0). The decrease of saturated FA content is mirrored by an increase in unsaturated FAs in the range of 40–90 %, mainly palmitoleic (16:1), oleic (18:1), and linoleic acid (18:2); the contribution of these three FA increases with increasing concentration of nZVI. The amount of palmitoleic acid (16:1) increases in cultivations with nZVI concentrations of 5–9 mg/L. The upper concentration (13 mg/L) induced in five species a slight decrease in the amount of palmitoleic acid, with the exception of *S. cerevisiae* in which the amount of this acid continued growing. As depicted, the maximum unsaturated FA content of all seven yeast species was observed when the concentration of nZVI was 13 mg/L. Compared with the control cultivation (without nZVI), the unsaturated FA content was 10–20 % higher. Addition of nZVI increased total lipid content by as much as 20 % in oleaginous yeasts (*Candida* sp.) and by as much as 100 % in non-oleaginous yeasts (*S. cerevisiae*). The content of unsaturated FA in oleaginous yeasts is usually 80–90 % of total FA. Fatty acids from *Candida* sp., *R. glutinis*, and *Y. lipolytica* are comprised mostly of monounsaturated FA, only *T. cutaneum* contains twice as much of linoleic acid as monounsaturated FAs. In non-oleaginous yeasts, *S. cerevisiae* and *K. polysporus* monounsaturated FA form 70–90 % of total FA; in *T. delbrueckii*, we observed about 50 % monounsaturated FA and 20 % diunsaturated FA out of total FA.

Cultivation with zero-valent iron nanoparticles

Besides limited nitrogen sources, several trace elements influence the growth and lipid accumulation of oleaginous yeasts. Addition of trace elements showed a positive effect on the growth and lipid accumulation by several strains of oleaginous yeast (Zhao et al. 2008; Zhu et al. 2008). Different metal ions (e.g., Cu^{2+}, Zn^{2+}, La^{3+}, etc.) could influence the activity of key enzymes participating in lipid accumulation (Jernejc and Legisa 2002).

The influence of the presence of iron and its valency (Fe^{2+} or Fe^{3+}, Fe^{2+} to Fe^{3+} intake equal to 5:1) or its availability is most apparent in the metabolism of triacylglycerols in the

Table 1 Fatty acid profile (%*w*/*w*) and total lipids of seven yeast strains cultivated with different Nanofer 25 concentrations

Yeast	Nano Fe (mg/L)	16:0 P	18:0 S	20:0 A	16:1 Po	18:1 O	18:2 L	Minor[a]	% lipids
Candida sp.	0[b]	22.4	10.7	4.1	6.9	29.8	25.0	1.1	22.4
	5	19.2	7.0	2.3	7.7	33.4	28.5	1.9	19.2
	9	17.6	2.0	1.4	10.7	35.0	31.0	2.3	17.6
	13	**16.9**	**1.6**	**0.5**	9.8	37.2	31.4	**2.6**	16.9
Kluyveromyces polysporus	0	7.4	0.9	0.9	59.1	28.7	2.7	0.3	7.4
	5	3.5	0.7	0.8	61.4	30.1	3.5	0.0	3.5
	9	3.2	0.6	0.7	58.5	32.8	3.9	0.3	3.2
	13	**1.9**	**0.6**	**0.5**	57.7	34.0	**5.1**	0.2	**1.9**
Rhodotorula glutinis	0	19.7	10.8	1.2	0.3	57.2	10.4	0.4	19.7
	5	14.1	7.4	0.7	0.9	62.7	13.6	0.6	14.1
	9	13.5	6.5	0.2	1.3	64.0	14.0	0.5	13.5
	13	**12.3**	**4.3**	**0.0**	**1.1**	65.9	**15.6**	**0.8**	**12.3**
Saccharomyces cerevisiae	0	14.5	4.1	3.7	45.8	28.6	0.0	3.3	14.5
	5	13.3	3.7	2.1	48.6	29.2	0.0	3.1	13.3
	9	12.1	2.3	1.2	51.6	29.6	0.0	3.2	12.1
	13	**10.3**	**1.9**	**0.2**	53.7	30.4	0.0	3.5	**10.3**
Torulospora delbrueckii	0	25.0	3.6	6.6	22.6	24.3	16.8	1.1	25.0
	5	20.8	2.8	4.7	25.9	26.9	17.9	1.0	20.8
	9	17.2	2.1	3.7	24.8	29.9	21.6	0.7	17.2
	13	**11.3**	**1.9**	**3.5**	23.4	**33.6**	**25.8**	**0.5**	**11.3**
Trichosporon cutaneum	0	17.0	3.8	1.7	2.6	26.1	48.8	0.0	17.0
	5	14.9	2.4	1.6	1.6	28.1	51.1	0.3	14.9
	9	13.8	1.1	1.4	1.0	29.3	53.1	0.3	13.8
	13	**10.5**	**0.5**	**1.0**	**0.8**	30.1	56.8	0.3	**10.5**
Yarrowia lipolytica	0	15.2	4.1	4.3	13.0	50.8	10.2	2.4	15.2
	5	13.4	3.1	2.6	8.0	55.7	15.0	2.2	13.4
	9	8.3	2.8	1.8	7.9	59.5	17.5	2.2	8.3
	13	**3.5**	**2.0**	0.9	**7.3**	64.4	**19.9**	2.0	**3.5**

Table entries in bold indicate marked change in FA or lipid content in the presence of nZVI

[a] Minor fatty acids, e.g., 14:0, 14:1, 15:0, 17:0, 17:1, etc., up to 0.3 % of total fatty acids

[b] Control, the yeast was cultivated without nZVI

fungus *Histoplasma capsulatum* and on the composition of individual fatty acids constituting the molecule (Zarnowski et al. 2008). Iron deficiency caused a 67 % increase in saturated fatty acids. No significant differences were found in the production of biomass between cultures growing in the presence of Fe^{2+} and Fe^{3+} while a significantly lower biomass production was observed in a control culture.

The effect of N and Fe limitations was studied with the aim to increase the proportion of saturated fatty acids in the yeast *Cryptococcus curvatus*; the Fe-limitation had no effect on biomass production (Hassan et al. 1996). A slight increasing tendency of the content of saturated fatty acids, particularly palmitic, stearic, and oleic acids was found between 5 and 25 h of cultivation under Fe limitation, whereas the content of linoleic and linolenic acids was reduced, e.g., linolenic acid content decreased from 40 to 30 % of total FAs between 5 and 25 h of culture. Although according to the authors "growth and lipid production, particularly stearic acid production, were not affected by Fe-limited condition", their data clearly show that the production of dienoic and trienoic fatty acids (L and Ln) has been negatively affected by Fe starvation.

Fe limitation was also studied in the yeast *R. glutinis* (Granger et al. 1993). Under these conditions, the content of α-linolenic acid was reduced, which is likely related to a decrease in the activity of desaturases whose activity depends on the presence of iron (Shanklin and Cahoon 1998).

Our results are fully in line with the above published data on the effect of iron on biomass production, total lipids, and fatty acid composition.

Previous studies (Philpott 2006) as well as our experiments show that Fe is an important trace element affecting primarily the quantitative composition of FAs. It is clearly seen to

Table 2 Percent content of lipids and saturated and unsaturated fatty acids in the total fatty acids in yeast strains

Yeast	Nano Fe (mg/L)	Sat	Monounsat	Polyunsat	Unsat	% lipids
Candida sp.	0[a]	37.4	37.6	25.0	62.6	4.6
	5	28.7	42.8	28.5	71.3	3.3
	9	21.1	47.9	31.0	78.9	5.3
	13	19.0	49.6	31.4	81.0	5.9
Kluyveromyces polysporus	0	9.4	87.9	2.7	90.6	5.1
	5	5.0	91.5	3.5	95.0	6.3
	9	4.5	91.6	3.9	95.5	7.1
	13	3.1	91.8	5.1	96.9	7.3
Rhodotorula glutinis	0	31.8	57.8	10.4	68.2	30.7
	5	22.3	64.1	13.6	77.7	30.7
	9	20.2	65.8	14.0	79.8	31.7
	13	16.6	67.8	15.6	83.4	31.2
Saccharomyces cerevisiae	0	25.1	73.1	1.8	74.9	4.3
	5	20.9	73.1	6.0	79.1	4.1
	9	16.6	76.0	7.4	83.4	9.3
	13	13.2	78.9	7.9	86.8	8.3
Torulospora delbrueckii	0	36.0	47.2	16.8	64.0	4.6
	5	28.9	53.2	17.9	71.1	5.1
	9	23.3	55.3	21.4	76.7	5.9
	13	16.9	57.3	25.8	83.1	6.3
Trichosporon cutaneum	0	22.5	28.7	48.8	77.5	48.9
	5	19.0	29.8	51.2	81.0	50.2
	9	16.4	30.3	53.3	83.6	53.7
	13	12.2	31.0	56.8	87.8	58.5
Yarrowia lipolytica	0	25.0	64.8	10.2	75.0	7.3
	5	20.2	64.8	15.0	79.8	5.8
	9	13.9	68.6	17.5	86.1	7.8
	13	7.2	72.9	19.9	92.8	7.4

[a] Control, the yeast was cultivated without nZVI

depend not only on whether the iron is present but also on its valence. The most beneficial for the cells are seen to be divalent iron ions, i.e., Fe^{2+} (Philpott 2006). Cultivation of yeast with zero-valent iron, i.e., nZVI, ensures a constant source of Fe^{2+} ions since the zero-valent iron is slowly oxidized ($Fe^0 \rightarrow Fe^{2+} \rightarrow Fe^{3+}$). This makes it possible to guarantee a substantially constant intake of Fe^{2+} by cells without the energy-consuming reduction of Fe^{3+} to Fe^{2+}. The concentration of nZVI in our experiments was based on literature data as the concentration of Fe^{2+} ion used in yeast cultivation is about 5 mg/L (Granger et al. 1993). The effects of various concentrations of nZVI on the growth of yeast species (see Supplements) are shown on the example of the growth curves (*K. polysporus*, *T. cutaneum*, and *Y. lypolytica*). These data show that the yeast grew at concentrations nZVI from 1.7 to 170 mg/L.

PCA was used to establish the taxonomic identity of the individual cultured strains of yeast, visualize the variability, and bring out the main patterns in the dataset. Figure 1 clearly shows that the main source of variability in the fatty acid composition is the fact that the strains belong to different taxons and the production of a fatty acid, i.e., its chemical structure, is governed by the respective strain. For instance, the yeast species with the best production of oleic acid are *R. glutinis* and *Y. lipolytica* and the production increases with increasing concentration of nZVI in the medium (upper left quadrant in Fig. 1). Likewise, *T. cutaneum*, which is the best studied yeast species in terms of production of linoleic acid, gives maximum production of this acid at 13 mg/L nZVI (lower left quadrant) whereas arachidonic acid production by *T. delbrueckii* decreases with increasing concentrations of the nZVI (lower right quadrant).

RDA was used to test directly the statistical significance of factors influencing fatty acid composition.

Polyunsaturated fatty acids (PUFA) are more susceptible to oxidation and are part of non-enzymatic antioxidant defense mechanism in cells (Richard et al. 2008). Therefore, the change in the abundance of PUFA in treated yeasts could be

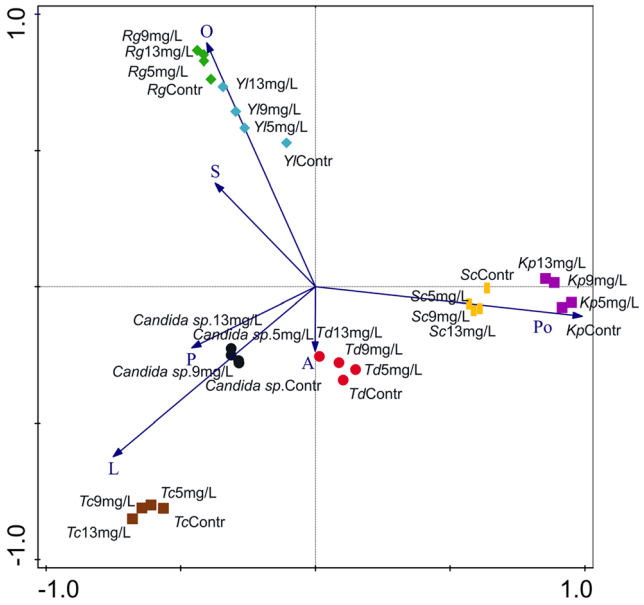

Fig. 1 Principal component analysis (PCA) of the relative proportions of fatty acids in seven strains cultivated at 5, 9, and 13 mg/L of nZVI. The first two canonical axes are plotted; they explained 94 % of the variability. For FA (*A*, *L*, *O*, *S*, *P*, and *Po*), for abbreviations, see Table 1. Strains *Kluyveromyces polysporus* (*Kp*), *Rhodotorula glutinis* (*Rg*), *Saccharomyces cerevisiae* (*Sc*), *Torulospora delbrueckii* (*Td*), *Trichosporon cutaneum* (*Tc*), *Yarrowia lipolytica* (*Yl*), and *Candida* sp.

connected with oxidative stress. The nZVI particles are also the reason of ROS formation and further development of oxidative stress (Ševců et al. 2011). The change in the composition of fatty acids in favor of PUFAs was described by Cipak et al. (2008) in the yeast *S. cerevisiae* exposed to oxidative stress conditions. The same change in fatty acid composition, which was accompanied by oxidative stress, was recorded by Kang et al. (2014) in *C. vulgaris* strain UTEX 265 as a reaction to the treatment by TiO_2 nanoparticles. Yilancioglu et al. (2014) reported that an increase of lipid accumulation in green alga *Dunaliella salina* was triggered by oxidative stress. The increase in abundance of PUFAs with nZVI concentration, as a response to ROS action, supports the effect of oxidative stress on lipid accumulation.

Oxidative stress

In this paper, the oxidative stress conditions, as a response to nZVI action, were checked by determining the quantity of lipid peroxidation products. Generally, the interaction of fatty acids with double bond in the molecule, especially PUFAs, with free oxygen radicals leads to lipid oxidation (Del Rio et al. 2005). The significantly higher level of TBARS, which was determined in the yeast *T. cutaneum* cultivated in medium amended with Nanofer 25 (13 mg/L), supported the oxidative action of nZVI (Table 3). Under nZVI treatment, a high amount of TBARS was noticed throughout the cultivation, unlike the control population. Therefore, the results indicate

Table 3 The effect of Nanofer 25 (nZVI; 13 mg/L) on the level of TBARS in *Trichosporon cutaneum*

	TBARS (μg/mg dry mass)	
Cultivation time (hours)	Control	With nZVI (13 mg/L)
24	21.87	63.22
48	2.09	20.87
72	1.21	31.07
96	7.41	45.95

Relative deviation is ±4 % as calculated from three replicates

that the higher degree of unsaturation in fatty acids, lipid accumulation as well as lipid peroxidation can be connected with oxidative stress conditions induced by the effect of nZVI.

According to the results of other authors (Chen et al. 2013; Gaensly et al. 2014), the concentration of minor elements such as Fe, Mg, Cu, Mn, and K has a substantial influence on the biomass, lipid content, and the percentage of the FA. Therefore, it is unlikely that the effect is caused by further increasing the concentration of iron; rather, the alleged effect may be caused by oxidative stress induced by nZVI.

Conclusion

In seven yeast species, zero-valent iron nanoparticles were found to exert a stimulatory effect on the production of biomass and lipids and a desirable change in fatty acids. Changes in lipid metabolism can be influenced by both an oxidative action of nZVI and by a sufficient amount of the desired ferrous ions generated from the zero-valent iron in the culture medium. The maximum biomass content was found in the majority of yeast at nZVI concentrations of 9 mg/L. The lipid content at nZVI concentrations of 5 to 13 mg/L was not substantially changed; further addition of nZVI caused changes in fatty acid composition and caused a higher proportion of unsaturated FAs.

Acknowledgments The research was supported by GACR 14-00227S, by Financial support from specific university research (MSMT No 20/2015), and by Competence Center TE01020218 of the Technology Agency of the Czech Republic.

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