

# Bioeffects of selenite on the growth of *Spirulina platensis* and its biotransformation

Zhi-Yong Li<sup>a,\*</sup>, Si-Yuan Guo<sup>b</sup>, Lin Li<sup>b</sup>

<sup>a</sup> College of Life Science and Biotechnology, Shanghai Jiaotong University, Shanghai 200240, China

<sup>b</sup> College of Food and Bioengineering, South China University of Technology, Guangzhou 510641, China

Received 6 June 2001; received in revised form 5 December 2002; accepted 19 January 2003

## Abstract

The bioeffects of selenium on the growth of *Spirulina platensis* and the selenium distribution were investigated. *S. platensis* was batch cultured in Zarrouk medium containing increasing concentrations of sodium selenite. The biotransformation characteristic of selenium was analysed by the determination of the detailed selenium distribution forms. At 35 °C, 315.2  $\mu\text{Em}^{-2}\text{s}^{-1}$ , sodium selenite concentrations below 400  $\text{mg l}^{-1}$  were found to stimulate algal growth, especially in the range of 0.5–40  $\text{mg l}^{-1}$ . However, above 500  $\text{mg l}^{-1}$  sodium selenite was toxic to this alga with the toxicity being related to the sulfite level in the medium. *S. platensis* was found to resist higher selenite by reducing toxic Se(IV) to nonsoluble Se(0). Selenium was accumulated efficiently in *S. platensis* during cultivation with accumulated selenium increasing with selenite concentration in the medium. It was demonstrated that inorganic selenite could be transformed into organic forms through binding with protein, lipids and polysaccharides and other cell components. The organic selenium accounted for 85.1% of the total accumulated selenium and was comprised of 25.2% water-soluble protein-bound, 10.6% lipids-bound and 2.1% polysaccharides-bound selenium. Among the organic fractions lipid possessed the strongest ability to accumulate Se (6.47  $\text{mg kg}^{-1}$ ). The 14.9% inorganic selenium in *S. platensis* was composed of Se(IV) (13.7%) and Se(VI) (1.2%).

© 2003 Elsevier Science Ltd. All rights reserved.

**Keywords:** *Spirulina platensis*; Selenium; Biotransformation; Batch culture

## 1. Introduction

The roles of selenium in the biosphere, both beneficial and deleterious, are gradually being determined (Bowie et al., 1996; Daniels, 1996). Many microorganisms are known to be able to use selenium (e.g., selenite, selenate or other forms) in their metabolism (Roux et al., 2001; Fleet-Stalder et al., 2000). As for microalgae, selenium has been shown to be an essential element for the growth of the marine diatom *Thalassiosira pseudonana* (Price et al., 1987), while a further survey of 27 species revealed 19 out of 27 species required selenium in low concentration for growth (Harrison et al., 1988). It has also been shown that the growth of some species of marine microalgae could be affected by high concentrations of selenite and selenate (Wong and Luis, 1991a). In the case of *Spirulina platensis*, although there was a previous

study (Cases et al., 1999) regarding Se-*Spirulina* nutrition, little is known about selenium enriched culture technique and the detailed selenium distribution within algal cells, especially the organic nature of selenium.

The essential trace mineral, selenium, is of fundamental importance to human health. As a constituent of selenoproteins, selenium has structural and enzymic roles (e.g., GSH-Px), in the latter context being best-known as an antioxidant and catalyst for the production of active thyroid hormone (Cases et al., 1999). Selenium is needed for the proper functioning of the immune system (Rayman, 2000), and appears to be a key nutrient in counteracting the development of virulence and inhibiting HIV progression to AIDS (Sandstrom et al., 1998). Selenium bioeffects are mainly involved in immune function, reproduction, mood, thyroid function, cardiovascular disease, cancer, viral infection, metal toxicity and other functions (Jr and Gray, 1998; Rayman, 2000). Recent evidence has reinforced the importance to health of adequate selenium status (Sun et al., 1998; Jochum et al., 1999; Reilly, 1998). Presently, supplement

\* Corresponding author. Fax: +86-21-54743348.

E-mail address: [zyli@sjtu.edu.cn](mailto:zyli@sjtu.edu.cn) (Z.-Y. Li).

of selenium from natural food materials is considered safer than directly ingesting inorganic selenium.

The cyanobacterium *S. platensis* is commercially available for human consumption. *Spirulina* represents one of the richest protein sources of plant origin (60–70%) and is a good source of vitamins and minerals although a low amount of selenium is found (from 0.01 to 0.04 mg kg<sup>-1</sup>) (Cases et al., 1999; Vonshak, 1997). *Spirulina* can be utilized for the production of health foods commanding a high market value because some chemicals are either unique to the alga or found at relatively high concentration, such as polyunsaturated fatty acid  $\gamma$ -linolenic acid (GLA), phycocyanin, myxoxanthophyl and zeaxanthin and other chemicals (Hirahashi et al., 2002; Premkumar et al., 2001; Vonshak, 1997). The simple cultivation technology and the high quality of its protein, as well as the absence of any toxic side effects (Salazar et al., 1998), favour large scale production. Moreover, the ability to control chemical composition by varying cultivation conditions (Morist et al., 2001) makes *Spirulina* the most easily Se-supplementable vegetable by way of the aquatic environment.

Although Cases et al. (1999) has studied the enhancement of GSH-Px activity by Se-rich *Spirulina*, the possible organic nature of selenium in *Spirulina* remains to be assessed. Meanwhile, in order to produce Se-rich *Spirulina* in a large scale, the effects of different concentrations of Se on the growth of *Spirulina* need to be investigated. The objective of this study was to investigate the effects of selenite on the growth of *S. platensis* and to thoroughly characterize the selenium distribution with practical emphasis on the organic nature.

## 2. Methods

### 2.1. Strain and cultivation

*S. platensis* was obtained from South China Normal University, Guangzhou, China, and was batch cultured in Zarrouk medium as described by Watanabe et al. (1995) containing increasing amounts of sodium selenite (determined by the added volume of 0.0578 mol l<sup>-1</sup> sodium selenite and its 1:10 and 1:1000 diluted solutions) under 35 °C, 315.2  $\mu\text{Em}^{-2}\text{s}^{-1}$  with selenium-free medium as the control. In this experiment, selenium accumulated in *S. platensis* was analysed by atomic absorption spectrometer (AAS) (HITACHI 180-80).

In order to find an ideal concentration range for selenite-stimulated culture of *S. platensis*, 5 concentration levels of selenite between 0 and 500 mg l<sup>-1</sup> were investigated at 35 °C and 315.2  $\mu\text{Em}^{-2}\text{s}^{-1}$  in this experiment. Biomass dry weight was determined every 24 h, where 10 ml cultures were filtered onto preweighed filter paper, washed with distilled water to remove

loosely bound selenium and dried at 60 °C to constant weight. All measurements were done in triplicate.

### 2.2. Isolation and purification of protein, lipids and polysaccharides

*S. platensis* cultured under 10 mg l<sup>-1</sup> sodium selenite in 3.3 was used to analyse selenium distribution in this experiment. *S. platensis* was harvested by the filtering method and freeze-dried to obtain algal powder. Subsequently protein, lipids and polysaccharides were isolated and purified respectively as follows: the algal powder was suspended in 0.1 mol l<sup>-1</sup> PBS buffer (pH = 7), frozen in liquid nitrogen for 10 min and then thawed in 40 °C water. The residues after centrifugation at 4000g for 10 min were treated as above for an additional two times. The protein containing supernatant was precipitated by 30–70% (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and the obtained crude protein eluted on a Sephadex G-100 column (16 × 50 cm) with 0.1 mol l<sup>-1</sup>, pH = 7 PBS at a rate of 0.5 ml min<sup>-1</sup>. The eluted protein solutions were collected and dialyzed against sterilized water, concentrated with polyglycol ( $M_r = 20000$ ) and finally freeze-dried. The total protein content was determined by a Kjeldahl method using a 6.25 conversion factor (Holme and Pech, 1998).

The algal powder was soaked in isopropylalcohol at a weight ratio of 1:100 for 24 h before centrifugation at 8000g for 20 min, and the solid obtained was resuspended in 1:1 (V/V) chloroform-isopropylalcohol for 48 h at a weight ratio of 1:100 (alga/chloroform-isopropylalcohol). The supernatant mixture after centrifugation (4000g, 10 min) was concentrated under reduced pressure. The crude lipids obtained were purified on a 3.5 × 15 cm column packed with silica gel, where non-polarlipids were eluted by chloroform and polarlipids with methanol at 15 ml h<sup>-1</sup>.

Polysaccharides were extracted from the algal powder with 80 °C sterilized water for 4 h. After centrifugation (4000g, 10 min), the residues were extracted again for an additional three times. After protein was removed by 1:5 (V/V) chloroform and 1:25 (V/V) butylalcohol, the combined supernatant after centrifugation (4000g, 10 min) was precipitated with 1:5 (V/V) industrial ethanol and CH<sub>3</sub>COONa for 24 h. The residue after centrifugation (4000g, 10 min) was washed with 95%. The residual crude polysaccharides were purified by eluting on a 2.0 × 92 cm DEAE-Sephadex A-25 column with 0.1 mol l<sup>-1</sup> NaCl solution at a rate of 15 ml h<sup>-1</sup>.

### 2.3. Determination of inorganic Se(IV) and Se(VI) and organic selenium

Selenium contents were determined spectrophotometrically by using a modification of the method of Kessi et al. (1999) with a 850 fluorimeter (HITACHI),

where  $\lambda_{\text{ex}} = 378.1$  nm,  $\lambda_{\text{em}} = 518$  nm, EX = 5 nm, EM = 5 nm. Selenium content could be determined by the linear relationship between the fluorescence intensity of the Se(IV)-2,3-diaminonaphthalene (DAN) complex at pH = 1–2 and Se(IV) concentration.

Total Se in alga, protein, lipids and polysaccharides was determined by digestion of the samples with perchloric acid and nitric acid at 60 °C followed by Se(VI) reduction to Se(IV) with 15% hydrochloric acid. Se(IV) content was determined directly using algal powder without the treatment as above. Se(VI) content was obtained from the Se(IV) content difference between the samples with and without reduction by 15% hydrochloric acid. Organic Se could be calculated from the content difference between total Se and the amounts of inorganic Se(IV) and Se(VI). All measurements were done in duplicate.

#### 2.4. Statistical analysis

Origin 5.0 was used to determine significant differences amongst the treatments. One-way ANOVA and Duncan's multiple range test were used to determine the significant difference at  $P < 0.05$ .

### 3. Results

#### 3.1. Bioeffects of sodium selenite on the growth of *S. platensis* and red-Se phenomenon

Sodium selenite has either stimulating or toxic effects on the culture of *S. platensis* depending on the Se levels in media (Fig. 1,  $P < 0.05$ ). Increasing the selenite concentration in the culture medium (e.g. higher than 500 mg l<sup>-1</sup>) led to a drastically lower cell concentration. In particular, cultures were found to turn red due to the occurrence of Se(0) during the stationary phase under higher selenite stress and the color would increase with selenite concentration. It was the first time to find *S. platensis* was able to reduce Se(IV) to Se(0).

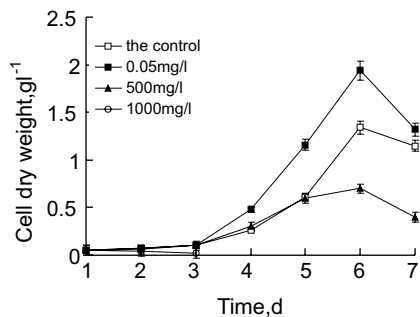


Fig. 1. Effects of sodium selenite on the growth of *S. platensis*.

#### 3.2. The relationship between sulfite level and the toxicity of higher concentration selenite

Under 500 mg l<sup>-1</sup> sodium selenite stress in medium, 550 and 1100 mg l<sup>-1</sup> sodium sulfite were fed in the culture to investigate their effects on the toxicity of selenite (Fig. 2,  $P < 0.05$ ). It was revealed that the existence of sulfite could decrease the toxicity of higher concentration selenite on *S. platensis*'s growth to some extent, especially at higher sulfite concentration.

#### 3.3. The enhanced culture of *S. platensis* by selenite addition and selenium accumulation

Although the growth of *S. platensis* could be stimulated by sodium selenite below 400 mg l<sup>-1</sup> (Fig. 3,  $P < 0.05$ ), the growth enhancement did not exhibit a positive relation with sodium selenite concentration. A peak increase was noted during the range of 0.5–40 mg l<sup>-1</sup>. At 10 mg l<sup>-1</sup> selenite, the maximum cell d.w. of *S. platensis* cultured in batch was 2.64 g l<sup>-1</sup> on the fifth day, which was 1.97 times better than that of the control (1.34 g l<sup>-1</sup>) ( $P < 0.05$ ). The upper limiting sodium selenite concentration was 400 mg l<sup>-1</sup> for the enhanced culture of *S. platensis*.

Selenium accumulation in *S. platensis* during the culture is illustrated in Fig. 4. It was demonstrated that selenium could be accumulated efficiently (Fig. 4) by *S. platensis* during the cultivation and selenium accumulation would increase with selenite concentration. For instance, at a selenite concentration in media of 10 and 400 mg l<sup>-1</sup>, respectively, levels of correspondingly 22 and 219 mg kg<sup>-1</sup> for selenium were obtained in *S. platensis*. These levels were considerably higher (137.5 and 1368.8 times, respectively) than that of the cultivation without selenite addition (0.16 mg kg<sup>-1</sup>) ( $P < 0.05$ ).

#### 3.4. The characteristics of selenium distribution in *S. platensis*

The selenium species that accumulated in *S. platensis* mainly consisted of organic Se and inorganic Se(IV),

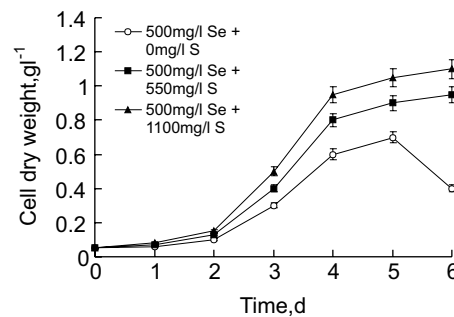


Fig. 2. Reduction of sodium selenite toxicity by sodium sulfite.

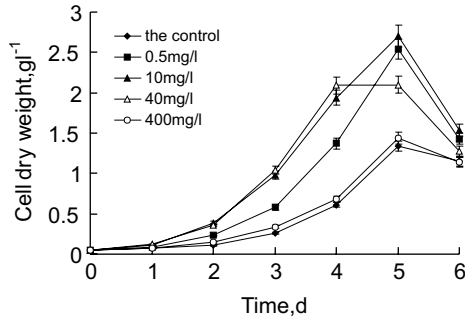


Fig. 3. Stimulation of sodium selenite to the growth of *S. platensis*.

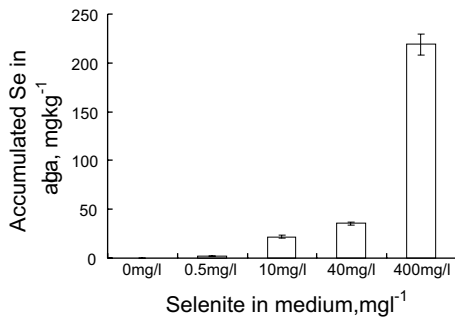


Fig. 4. Selenium accumulation in *S. platensis*.

Se(VI) at levels of 85.1%, 13.7% and 1.2%, respectively (Fig. 5,  $P < 0.05$ ) indicating that most of the selenite was bio-transformed into organic Se during the culture. The water-soluble protein-bound selenium was estimated at 14% of the total organic selenium, and based on its percentage in total protein (56% of algal dry wt) 42.6%, the total protein-selenium was estimated at approximately 25.2%. In addition to this, 10.6% selenium was bound with lipids and 2.1% with polysaccharides (Fig. 6,  $P < 0.05$ ). The superfluous 47.2% perhaps bound with the other ligands such as free amino acids, polypeptides, or other similar compounds.

Among water-soluble protein, lipids and polysaccharides, the ability to bind with Se was compared in Fig. 7. Although protein-bound Se was the major form of organic Se (25.2% of the total accumulated Se), lipids

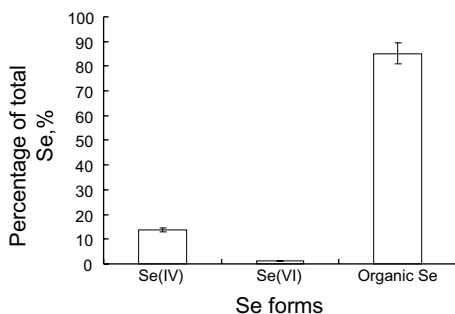


Fig. 5. Selenium forms in *S. platensis*.

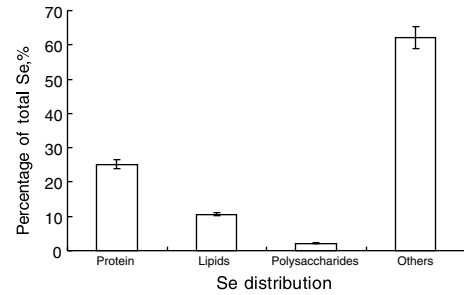


Fig. 6. Distribution of organic Se in biochemical components in *S. platensis*.

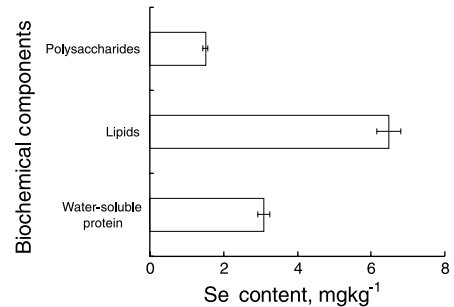


Fig. 7. Comparison of the Se-binding ability among water-soluble protein, lipids and polysaccharides.

had the greatest ability to integrate with Se:  $6.47 \text{ mg kg}^{-1}$ . Followed by water-soluble protein  $3.09 \text{ mg kg}^{-1}$  and polysaccharides  $1.51 \text{ mg kg}^{-1}$  ( $P < 0.05$ ).

The detailed distribution of selenium among water-soluble protein, lipids and polysaccharides is listed in Table 1. Protein integrated with chlorophyll (part A) was the main binding position for Se in protein (54.23% of the total protein-Se). As for lipids, the majority of Se was bound to polar-lipids (73.43% of the total lipids-Se). For polysaccharides, the amounts of Se in parts A and B were only 84.28% of the total polysaccharides-bound Se

Table 1  
The detailed distribution of Se in water-soluble protein, lipids and polysaccharides

	Se percentage (%)
<i>Water-soluble protein</i>	
Part A	$54.23 \pm 0.06$
Part B	$35.75 \pm 0.05$
Part C	$7.52 \pm 0.03$
<i>Lipids</i>	
Polar-lipids	$73.43 \pm 0.03$
Nonpolar-lipids	$26.55 \pm 0.05$
<i>Polysaccharides</i>	
Part A	$38.86 \pm 0.04$
Part B	$45.42 \pm 0.06$
Others	$15.71 \pm 0.07$

Note: values are means  $\pm$ SD ( $n = 3$ ).

indicating that potentially there were other carbohydrates binding Se.

#### 4. Discussion

Based on this study, selenium is an essential trace element at low concentrations, as well as a toxicant at high concentrations, to *S. platensis*, which is consistent with other trace elements. These observations suggest that a suitable selenite concentration range must be selected for culture enhancement of *S. platensis*. Based on our results, it is suggested that, at 35 °C, 315.2  $\mu\text{Em}^{-2}\text{s}^{-1}$ , a sodium selenite concentration range of 0.5–40  $\text{mg l}^{-1}$  is most suitable for selenium enriched culture of *S. platensis*.

It appears that the stimulating effects of selenite on the growth of *S. platensis* may derive from the increased level of Se enzyme resulting in the more effective removal of free radicals and consequently a reduction in the rate of algal cell decline. In the case of selenite toxicity, a high concentration of selenium may cause changes in the nucleus, lipids, vacuoles, nitrogen and carbon contents. Wong and Luis (1991b) have suggested that energy-transduction systems may be severely affected by selenium toxicity, which may lead to substantial decreases or even elimination of storage products and major reductions in growth.

*S. platensis* is able to resist selenite through accumulation in cells at a low concentration of selenite and reduce selenite to element selenium at a higher concentration since Se(0) is poorly soluble and thus less toxic than selenite. The toxicity decrease of higher concentration selenite in the presence of sulfite suggested there is competition between selenium and sulphur metabolisms in *S. platensis*. Previously it has been demonstrated that some microorganisms, such as *Rhodospirillum rubrum* (Kessi et al., 1999), *Rhodobacter sphaeroides* (Fleet-Stalder et al., 2000) and *Ralstonia metallidurans* CH34 (Roux et al., 2001), are able to reduce selenite or selenite to Se(0) or other forms. However this was the first time a reduction process during selenium metabolism in *S. platensis* was observed, as well as an apparent toxicity of selenite in relation to the sulfite level.

Obviously, *S. platensis* could bioaccumulate Se efficiently during the culture and the accumulation amount increased with selenite concentration. Our results revealed that the majority of inorganic Se was changed into organic Se (85.1% of the total accumulated Se) by integrating with the algal bioligands such as protein, lipids, polysaccharides and other algal components, where about 25.2% total accumulated Se was integrated with protein. The research of Bottino et al. (1984) on *Dunaliella primolecta*, *Chlorella* sp. and *Porphyridium cruentum* indicated there were both free- and protein-bound seleno-amino acids in these microalgae. In gen-

eral, Se may follow sulphur metabolism when it is being incorporated into amino acids and protein. Thus, selenium incorporation may involve the use of the sulphur enzymatic metabolism resulting in the replacement of some of the sulphur by selenium in both free amino acids and protein, which has been demonstrated by the presence of seleno-analogues of the sulphur amino acids during selenium accumulation (Wrench, 1978). This conclusion was also supported by the results of our work concerning the toxicity reduction to some extent of high concentration selenite in the presence of sulfite. In addition, some changes in IR spectrum before and after selenium accumulation in *S. platensis*, e.g., the percentage transmittance decrease or disappearance at 628  $\text{cm}^{-1}$  (S–S) and 1168  $\text{cm}^{-1}$  (S=O) (data not shown), also supported the concept of selenium accumulation instead of sulphur in *S. platensis*.

In addition to protein, lipids and polysaccharides were also involved in the accumulation of Se. Among these components lipid has the highest Se-bound efficiency (6.47  $\text{mg kg}^{-1}$ ). Gennity et al. (1984) has suggested that lipid-associated selenium was not metabolically incorporated. Selenium was mainly bound with polar-lipids (73.4% of the total lipids-bound Se) indicating that selenium was probably noncovalently bound to the lipids. In the case of polysaccharides, according to the distribution of selenium in the different parts of polysaccharides, selenium is perhaps mainly integrated with those polysaccharides near the algal cell wall, e.g. peptidoglycan and other components.

Among the 85.1% organic Se, 47.2% were superfluous Se that perhaps was bound with free amino acids, polypeptides, carbohydrates and other components. Consequently it was obvious that *S. platensis* can adopt various strategies to integrate with Se with the biochemical components. The detailed selenium distribution in the ligands besides protein, lipids and polysaccharides needs to be studied more in depth. Since only 14.9% Se was in inorganic form, most of selenium could be taken up in *S. platensis* by active process other than physical adsorption. This is a beneficial mechanism for the improvement of the bioeffects of *S. platensis*.

#### 5. Conclusions

This research work has demonstrated that, with sodium selenite addition into the culture media of *S. platensis*, both the growth rate and magnitude of organic selenium content can be enhanced. This also suggests that a selenium enriched health food with notable market value may be produced efficiently as a *Spirulina* powder as well as selenium enriched protein, lipids and polysaccharides and other components.

## Acknowledgements

We thank the support of the Postdoctoral Research Fund from the Committee of Science and Technology, Shanghai, PR China. We are grateful to Prof. Steve McAnally very much for his review of the language.

## References

- Bottino, N.R., Bank, C.H., Irgolic, B.K., 1984. Selenium containing amino acids and proteins in marine algae. *Phytochemistry* 23, 2445–2452.
- Bowie, G.L., Sanders, J.G., Riedel, G.F., Gilmour, C.C., Breitbart, D.L., Cutter, G.A., Porella, D.B., 1996. Assessing selenium cycling and accumulation in aquatic ecosystems. *Water Air Soil Pollut.* 90, 93–104.
- Cases, J., Puig, M., Caporiccio, B., Baroux, B., Baccou, J.-C., Besancon, P., Rouanet, J.-M., 1999. Glutathione-related enzymic activities in rats receiving high cholesterol or standard diets supplemented with two forms of selenium. *Food Chem.* 65, 207–211.
- Daniels, L.A., 1996. Selenium metabolism and bioavailability. *Biol. Trace Elem. Res.* 54, 185–199.
- Fleet-Stalder, V.V., Chasteen, T.G., Pickering, I.J., George, G.N., Prince, R.C., 2000. Fate of selenate and selenite metabolized by *Rhodobacter sphaeroides*. *Appl. Environ. Microbiol.* 66, 4849–4853.
- Gennity, J.M., Bottino, N.R., Zingaro, R.A., Wheeler, A.E., Irgolic, K.J., 1984. The binding of selenium to the lipids of two unicellular marine algae. *Biochem. Biophys. Res. Commun.* 118, 176–182.
- Harrison, P.J., Yu, P.W., Thomason, P.A., Price, N.M., Philips, D.J., 1988. Survey of selenium requirements in marine phytoplankton. *Mar. Ecol. Prog. Ser.* 47, 89–96.
- Hirahashi, T., Matsumoto, M., Hazeki, K., Saeki, Y., Ui, M., Seya, T., 2002. Activation of the human innate immune system by *Spirulina*: augmentation of interferon production and NK cytotoxicity by oral administration of hot water extract of *Spirulina platensis*. *Int. Immunopharmacol.* 2, 423–434.
- Holme, D.J., Pech, H., 1998. *Analytical Biochemistry*. Addison Wesley Longman, New York, pp. 388–390.
- Jochum, F., Jerwolbeck, K., Meinhardt, H., 1999. Is there any health risk of low dietary selenium in PKU-children? *Nutrition Research* 19 (3), 349–360.
- Jr, G.F.C., Gray, W.P., 1998. Chemopreventive agents: selenium. *Pharmacol. Ther.* 79, 179–192.
- Kessi, J., Ramuz, M., Wehrli, E., Spycher, M., Bachofen, R., 1999. Reduction of selenite and detoxification of elemental selenium by the phototrophic bacterium *Rhodospirillum rubrum*. *Appl. Environ. Microbiol.* 65, 4734–4740.
- Morist, A., Montesinos, J.L., Cusido, J.A., Godia, F., 2001. Recovery and treatment of *Spirulina platensis* cells cultured in a continuous photobioreactor to be used as food. *Process Biochem.* 37, 535–547.
- Premkumar, K., Pachiappan, A., Abraham, S.K., Santhiya, S.T., Gopinath, P.M., Ramesh, A., 2001. Effect of *Spirulina fusiformis* on cyclophosphamide and mitomycin-C induced genotoxicity and oxidative stress in mice. *Fitoterapia* 72, 906–911.
- Price, N.M., Thompson, P.A., Harrison, P.J., 1987. Selenium: an essential element for growth of the coastal marine diatom *Thalassiosira pseudonana*. *J. Phycol.* 23, 1–9.
- Rayman, M.P., 2000. The importance of selenium to human health. *Lancet* 356, 233–241.
- Reilly, C., 1998. Selenium: a new entrant into the functional food arena. *Trend in Food Technol.* 9, 114–118.
- Roux, M., Sarret, G., Pignot-Paintrand, I., Frontecave, M., Coves, J., 2001. Mobilization of selenite by *Ralstonia metallidurans* CH34. *Appl. Environ. Microbiol.* 67, 769–773.
- Salazar, M., Martinez, E., Madrigal, E., Ruiz, L.E., Chamorro, G.A., 1998. Subchronic toxicity study in mice fed *Spirulina maxima*. *J. Ethnopharmacol.* 62, 235–241.
- Sandstrom, P.A., Murray, J., Folks, T.M., Diamond, A.M., 1998. Antioxidant defenses influence HIV-1 replication and associated cytopathic effects. *Free Radical Biol. Medicine* 24, 1485–1491.
- Sun, Y., Ha, P.C., Butler, J.A., Ou, B.R., Yeh, J.Y., Whanger, P., 1998. Effect of dietary selenium on selenoprotein W and glutathione peroxidase in 28 tissues of the rat. *J. Nutr. Biochem.* 9, 23–27.
- Vonshak, A., 1997. *Spirulina platensis (Arthrospira)*: Physiology, Cell-biology and Biotechnology. Taylor & Francis Ltd., London, pp. 175–212.
- Watanabe, Y., Noue, J., Hall, D.O., 1995. Photosynthetic performance of a helical tubular photobioreactor incorporating the cyanobacterium *Spirulina platensis*. *Biotech. Bioeng.* 47, 261–269.
- Wong, D., Luis, O., 1991a. Effects of selenite and selenate toxicity on the ultrastructure and physiology of three species of marine microalgae. *Can. J. Fish. Aquat. Sci.* 48, 1201–1211.
- Wong, D., Luis, O., 1991b. Effects of selenite and selenate on the growth and motility of seven species of marine microalgae. *Can. J. Fish. Aquat. Sci.* 48, 1193–1200.
- Wrench, J.J., 1978. Selenium metabolism in the marine phytoplankters *Tetraselmis tetrahele* and *Dunaliella minuta*. *Mar. Biol.* 49, 231–236.