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Biosorption study of magnesium, zinc, iron and selene in *Spirulina platensis* high concentration crops

Estudo da biossorção de magnésio, zinco, ferro e selênio em cultivos de alta concentricão de *Spirulina platensis*

Estudio de biosorción de magnesio, zinc, hierro y seleno en cultivos de alta concentración de *Spirulina platensis*

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Abstract

Mineral biosorption is a technique that can bring significant gains to the production of functional biomass. The results guarantee economy (due to the absence of mineral addition in dry biomass), minimization of time and a step by step elimination in the production process. The aim of the present work was to study the incorporation of zinc, magnesium, iron and selenium in the biomass of *Spirulina platensis* through the application of central composite factorial designs. The experiments were performed in a vegetative greenhouse in 10-liter photobioreactors, aiming the maximum absorption of the minerals over five days. Significant influence was observed in the incorporation of magnesium (410 mg.100^{-1}g) and zinc (34.84 mg.100^{-1}g). It was also observed in the incorporation of Fe (73.5 mg.100^{-1}g) and selenium (1,738.81 mg.100^{-1}g), showing the potential use of the technique to incorporate these minerals in *Spirulina platensis* biomass and ensuring wide application of this raw in foods and supplements.

Keywords: Biofixation; Enrichment; Photobioreactors; *Spirulina platensis*.

Resumo

A biossorção de minerais é uma técnica que pode trazer ganhos significativos na produção de biomassa funcional. Os resultados podem garantir economia (por não adicionar minerais na biomassa seca), minimização do tempo e eliminação de etapas no processo produtivo. O objetivo do presente trabalho foi estudar a incorporação de zinco, magnésio, ferro e selênio na biomassa de *Spirulina platensis* por meio da aplicação de Planejamento Fatorial Composto Central. Os experimentos foram conduzidos em casa de vegetação em fotobiorreatores de 10 litros, tendo por objetivo a biossorção máxima dos minerais em cinco dias. Foi observada influência significativa na incorporação de magnésio (410 mg.100^{-1}g) e zinco (34,84 mg.100^{-1}g). Também foi observado na incorporação de Fe (73,5 mg.100^{-1}g) e selênio (1.738,81 mg.100^{-1}g), evidenciando o potencial de uso da técnica para incorporar esses minerais na biomassa de *Spirulina platensis* e garantir ampla aplicação desta matéria prima em alimentos e suplementos.

Palavras-chave: Biofixação; Enriquecimento; Fotobiorreatores; *Spirulina platensis*.

Resumen

La biosorción de minerales es una técnica que puede aportar importantes beneficios a la producción de biomasa funcional. Los resultados garantizan economía (debido la ausencia de adición de minerales en la biomasa seca), minimización de tiempos y eliminación etapas en el proceso productivo. El objetivo de este trabajo fue estudiar la incorporación de zinc, magnesio, hierro y selenio en biomasa de *Spirulina platensis* mediante la aplicación de Diseño Factorial Compuesto Central. Los experimentos se realizaron en invernadero vegetativo y fotobiorreactores de 10 litros, con el objetivo de lograr la máxima absorción de los minerales durante cinco días. Se observó una influencia significativa en la incorporación de magnesio (410 mg.100^{-1}g) y zinc (34.84 mg.100^{-1}g). También se observó en la incorporación de Fe (73,5 mg.100^{-1}g) y selenio (1.738,81 mg.100^{-1}g), mostrando el uso potencial de la técnica para incorporar estos minerales en la biomasa de *Spirulina platensis* y asegurar una amplia aplicación de esta materia prima en alimentos y suplementos.

Palavras clave: Biofijación; Enriquecimiento; Fotobiorreactores; *Spirulina platensis*.

1. Introduction

Besides being valued as food, microalgae have been also considered as a source of essential bioactive compounds for organisms. They provide pigments such as β-carotene or astaxanthin, polyunsaturated fatty acids, and almost all essential vitamins. Over the last three decades, the microalgae industry has gained importance due to its use in different fields of biotechnological process (Spolaore et al., 2006).

According to Chen et al., (2006), *Spirulina platensis* is a blue-green microalga that contains photosynthetic pigments. Chlorophyll, lutein, β-carotene, phycocyanin and allophycocyanin are the major bioactive components. It is a cyanobacteria rich in protein (65 % to 70 %), superior to any other natural food source. It contains vitamins, minerals, polyunsaturated fatty acids, essential amino acids and other nutrients (Li et al., 2003). The cell walls of microalgae are mainly made up of polysaccharides, proteins and lipids. They contain many functional groups (such as carboxylate, hydroxyl, thiol, sulfonate, phosphate, amino and the imidazole groups) and they can form coordination complexes with metal cations (Gong et al., 2005) and these functional groups are able to interact with metal ions in aqueous solutions (Saygideger et al., 2005).

Spirulina is classified by the FDA (Food and Drug Administration) as GRAS (Generally Recognized as Safe), which allows its use as food without causing health risks or harm (Arai, 1996). Chen et al., (2006) mentions that the biomass of *Spirulina platensis* has been commercialized in several countries and its components are used as nutrients for animal and human consumption. Fox, (1996) mentions that *Spirulina platensis* can be used as a food supplement in the fight against malnutrition, weight reduction, decreasing glucose levels in people with diabetes mellitus and with a decrease of renal toxicity. Karkos et al., (2008) in his review describes the potential potential for the fight against cancer, viruses and diabetes.

Currently *Spirulina* is being used as a food supplement and source of bioactive compounds in the food, agricultural, pharmaceutical, medical and cosmetic sectors. Its potential and the large number of health benefits are still being revealed (Reboleira et al., 2019). Melo et al., (2020) state that the incorporation of *Spirulina platensis* biomass in the studied foods increases their nutritional potential without affecting the sensory aspects.

Chen et al., (2006) reported that *Spirulina platensis* behaves as a good support for selenium transformation. In addition, it presents as an efficient absorption of minerals such as copper, chromium III, iodine (Saeid et al., 2013; Molnár et al., 2013).

Therefore, this work aimed to evaluate the rapid incorporation of zinc, magnesium, selenium and iron in cultures of high cellular Spirulina platensis concentration, aiming to obtain potential raw material for use in food formulations with greater usefulness.

2. Methodology

2.1 Microalgae Maintenance

Spirulina platensis was provided by the company Brasil Vital, located in Anápolis, Goiás (Latitude 16° 38’ South, Longitude 48° 82’ W, 1419 m). It was kept in test tubes with 2 ml of used in the absorption system at 23ºC, pH 9.5, during 11 hours of light and 13 hours of darkness, under illumination of 1500 lux (Melo et al., 2015).

2.2 Medium and absorption system

The growing medium was developed in laboratory (Melo et al., 2015), where the salt concentrations were maintained at high levels, due to the need for osmotic pressure between the medium and the cells. The growing medium was composed of: sodium bicarbonate (9.07 $g.L^{-1}$), NaCl (7.2 $g.L^{-1}$), NaOH (0.48 $g.L^{-1}$), K_2SO_4 (0.27 $g.L^{-1}$), $CaCl_2$ (0.1 $g.L^{-1}$), urea (0.02 $g.L^{-1}$), $FeSO_4$ (0.005 $g.L^{-1}$), $MgSO_4$ (0.19 $g.L^{-1}$), citric acid (0.01 $g.L^{-1}$), mono-ammonium-phosphate (0.19 $g.L^{-1}$) and 0,05 $mL.L^{-1}$ of trace elements solution with: H_3BO_3 (5.0 $g.L^{-1}$), $ZnSO_4$ (20.0 $g.L^{-1}$), $MnCL_2$ (2.0 $g.L^{-1}$), $CuSO_4$ (0.5 $g.L^{-1}$), Na_2SeO_3 (0.2 $g.L^{-1}$), MoO_3 (0.1 $g.L^{-1}$). Two experimental designs were used for the biosorption of zinc and magnesium, and the other one, iron and selenium. In the first one, there was a variation in the concentration of zinc sulfate and magnesium sulfate and in the second, the concentration of iron sulphate heptahydrate and sodium selenite varied. Both experiments followed a Central Composite Design, at two levels with two variables, three central replicates, four tests for a linear model, four tests orthogonally distributed (axial points) at a distance of ± 1,41 from the central point, totaling eleven experiments.

For the first design, the incorporation responses of zinc and magnesium in the biomass were quantified and the incorporation responses of iron and selenium were quantified for the second design.

The cultivation system was developed specifically for the conduction of the present study. First, a metal structure was built for fixing the stirring system, driven from a motor coupled to a speed reducer in order to maintain the rotation at 60 rpm. Round polyethylene containers of 46 cm in diameter and 10 liters in volume were used as open photo-bioreactors.

The photo-bioreactors were installed inside a vegetative greenhouse to maintain an adequate microclimate of temperature, humidity and luminosity for the conduction of the experiments.

The experiments were performed over 6 days at the Agronomy School of the Federal University of Goiás (Latitude 16° 59’ South, Longitude 49° 27’ W, 11343 m). The initial concentration of biomass was fixed at 5 $g.L^{-1}$, since the objective was the incorporation of minerals in the biomass and not the cellular multiplication.

The absorbance measurement was performed daily in a spectrophotometer at 620 nm, in order to determine if the high concentrations of the mineral sources would damage the maintenance of the initial concentration of *Spirulina platensis.*

2.3 Collection and the determination of minerals

After the end of the experiments, the photo-bioreactors were disconnected and the biomass was collected in filters with 30 μm of porosity. Subsequently, the filtered biomass was dried in a vacuum oven at 55°C for 24 hours. Then, the material was ground and packed for mineral analysis.

Zinc, magnesium, selenium and Iron analysis were performed by the Atomic Absorption Spectrophotometry methodology according to IAL, (2008).

2.4 Evaluation of Results

The results were analyzed by the software *Statistica* for Windows 7.0, with univariate analysis of variance and response surface technique.

3. Results and Discussion

It was observed that the cellular concentration of all experiments did not present significant difference (p-value < 0.1), during the six days that the strain of *Spirulina platensis* remained in the incorporation medium. Although not significant, a slight increase in biomass concentration was observed (in experiments with zinc and magnesium incorporation), but did not cause damage during the biofixation process.

Over the last years, some studies have been performed with the aim of observing the incorporation of minerals in *Spirulina* biomass. Some studies were reported by Li et al., (2003), Chen et al., (2005), Sahin et al., (2013), Saeid et al., (2013) and Molnár et al., (2013), where they found significant results for mineral biosorption.

Based on Table 1, the matrices of the experimental design with its codified and real values verify that the highest values obtained for the incorporation of magnesium were in the experiments 2a and 9a, where the highest levels were used for magnesium sulphate (+1 e +alfa, respectively). The lowest response was obtained for experiments 10a (lower level for zinc sulfate).

Table 1. Actual and coded matrix of the Central Composite Design 2^2 used for the incorporation of zinc and magnesium in *Spirulina platensis* biomass. Exp: Experiment; IM: Incorporation medium; BM: Biomass.

Exp	$MgSO_4$ in the IM ($mg.L^{-1}$)	$ZnSO_4$ in the IM ($mg.L^{-1}$)	Mg in BM ($mg.100^{-1}g$)	Zn in BM ($mg.100^{-1}g$)
1a	(-1)1,000	(-1)10	370.0	32.14
2a	(+1)2,000	(-1)10	410.0	31.05
3a	(-1)1,000	(+1)20	380.0	34.84
4a	(+1)2,000	(+1)20	400.0	32.10
5a	(0)1,500	(0)15	400.0	33.18
6a	(0)1,500	(0)15	390.0	32.41
7a	(0)1,500	(0)15	390.0	33.69
8a	($-\alpha$)790	(0)15	360.0	32.33
9a	($+\alpha$)2,210	(0)15	410.0	33.58
10a	(0)1,500	($-\alpha$)7.9	350.0	31.55
11a	(0)1,500	($+\alpha$)22.1	380.0	34.22

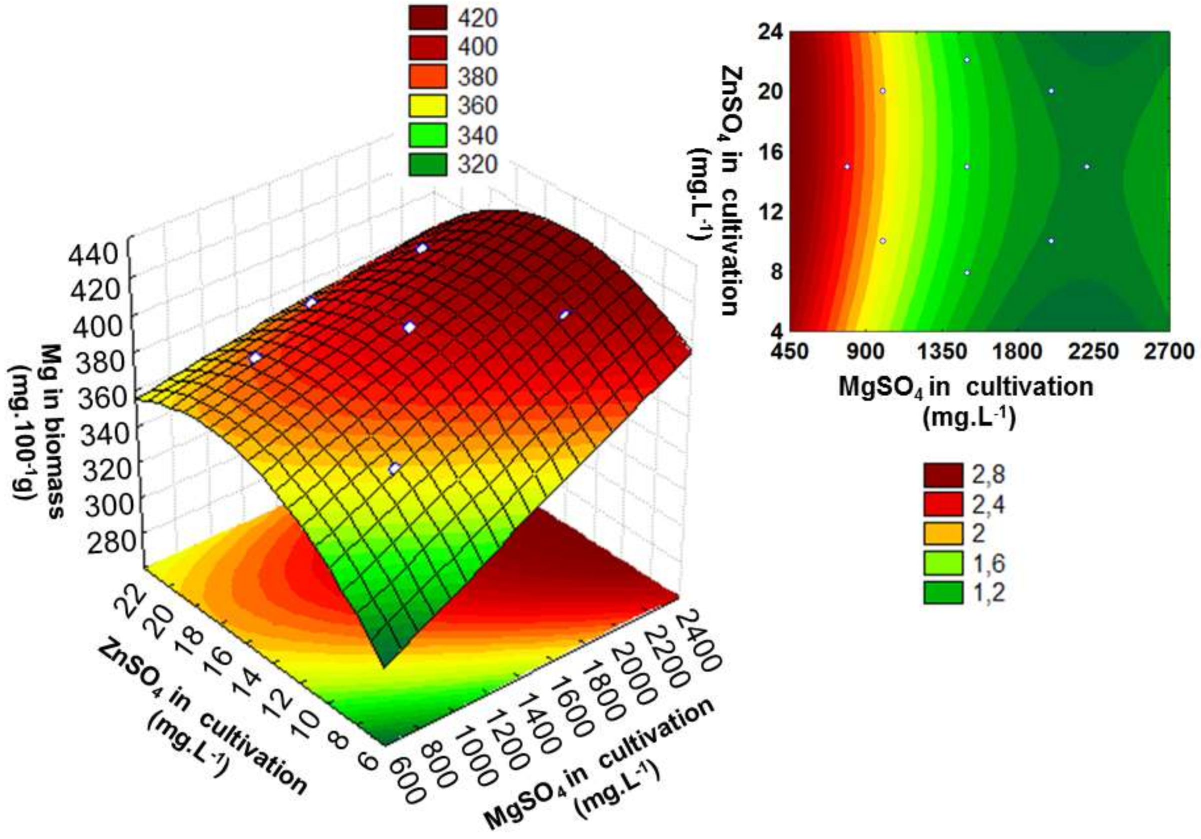
Source: Authors.

When zinc concentration is doubled, there is little change in the response variable (370 to 380 $g.L^{-1}$). In experiments 2a and 4a the response presents higher values because there is a higher concentration of magnesium sulphate in the incorporation medium when compared to experiments 1a and 3a. The similarity obtained in the central point responses (experiments 5a, 6a and 7a) show the repeatability quality of the experiments. In this experimental design, a significance level of 90 % was considered (p-value < 0.1). From the results obtained, a multiple regression, obtaining a mathematical model capable of predicting the absorption of magnesium (mg.100^{-1}g) against variations of magnesium sulphate and zinc sulphate ($R^2 = 80.47$ %) was developed, according to Equation 1.

$$[Mg] = 393.33 + 16.33 \times Mg_2SO_4 - 0.41 \times (Mg_2SO_4)^2 + 5.30 \times Zn_2SO_4 - 10.41 \times (Zn_2SO_4)^2 - 5.00 \times Mg_2SO_4 \times Zn_2SO_4 \qquad (1)$$

Figure 1 presents the response surface based on Equation 1 of the complete model. It can be observed that the region with the highest magnesium incorporation in the biomass occurs at the highest levels of Mg_2SO_4 and at the central levels of Zn_2SO_4. This fact can be justified by the proximity of the values from the central points of the experiments with the highest responses.

Figure 1. Response surface of the incorporated magnesium concentration (a) and contour curves of the percentage of incorporated magnesium (b) in Spirulina platensis biomass.



Source: Authors.

The lower biosorption indexes of magnesium can be observed in the lower levels of the two variables (experiments 8a and 10a). The interaction term is not significant. The experiment verified that magnesium sulfate could be analyzed individually where its effect has a positive value as a contribution in the response variable. When this variable is in the medium, it increases the absorption of magnesium by *Spirulina platensis.*

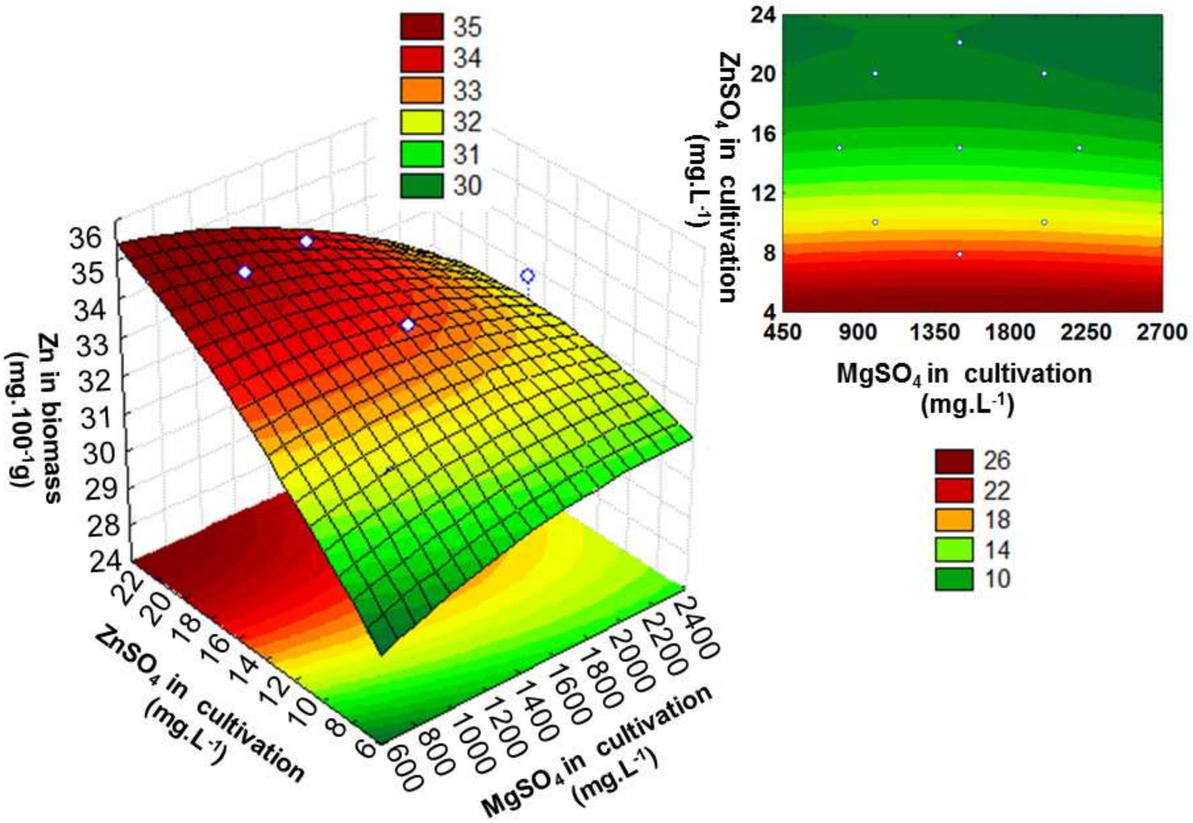
Magnesium is an essential element for the production of microalgae biomass. The magnesium content in microalgae varies between 0.35 % and 0.7 % (Tokuşoglu & üUnal, 2003), however a content of up to 7.5 % can be found in some species (Grobbelaar, 2004). Magnesium participates in vital cellular processes, such as ATP reactions to fix carbon and is an activator of several important enzymes. It is also a constituent of the photosynthetic apparatus and, in particular, of chlorophylls (Hopkins & Hüner, 2009). While few studies are reported about the absorption of magnesium sources in biomass, many studies have been carried out to study zinc absorption. An important aspect observed in this study is that the concentration of zinc sulfate provided a negative contribution. Thus, a lower concentration of this salt and a higher concentration of magnesium sulfate are suggested.

Considering zinc incorporation as a variable response, we showed that the lower biosorption rates of this mineral were found in experiments 2a and 10a (-α and -1, respectively). The greater biosorption was in experiment 3a (level +1 for $ZnSO_4$ and level -1 for $MgSO_4$). When comparing the experimental responses between experiments 3a and 11a, it was verified that with the increase of 50 % in the concentration of magnesium sulfate (from level -1 to level 0), the response had an increase of only 1.88 %, while the zinc concentration increased from +1 to +α. Therefore, it can be inferred that the concentration of magnesium sulfate does not have a big effect on the incorporation of zinc in *Spirulina platensis* biomass. Besides, the inclusion of 20 % more of zinc salt (20 $g.L^{-1}$ to 22.2 $g.L^{-1}$) does not provide a significant increase in zinc incorporation. From the results obtained, a multiple regression, obtaining a mathematical model was obtained capable of predicting the absorption of zinc ($mg.100^{-1}g$) against variations of magnesium sulphate and zinc sulphate (R^2 = 63 %), according to Equation 2.

$$[Zn] = 33.09 \quad 0.26 \times Mg_2SO_4 \quad 0.16 \times (Mg_2SO_4)^2 + 0.94 \times Zn_2SO_4 \quad 0.20 \times (Zn_2SO_4)^2 \quad 0.41 \times Mg_2SO_4 \times Zn_2SO_4 \qquad (2)$$

It was observed that only the linear term of zinc sulfate is significant at the level of 10 %. So its effect has a positive contribution in the incorporation of this mineral. Figure 2 shows the response surface based on Equation 2 of the complete model. It is observed that the region that presented the best zinc incorporation responses in the biomass were those in experiments 3a and 11a. In this design, the quality of repeatability of the experiments is verified according to the proximity of the values obtained at the central points.

Figure 2. Response surface of the incorporated zinc concentration (a) and contour curves of the percentage of incorporated zinc (b) in Spirulina platensis biomass.



Source: Authors.

Zinc is an essential micronutrient for algae growth. Its deficiency leads to low growth and low dry weight, but high concentrations can decrease cell division, motility, total chlorophyll content, the proportion of carotenoids, chlorophyll and ATPase activity (Omar, 2002). Carbonic anhydrase contains zinc as a prosthetic group, this new isoform expression suggests a phenomenon that, with the gradual addition of zinc, the carbon metabolism system improves (Palanisami et al., 2013).

Studies performed by Saeid et al., (2013) show a low zinc absorption (approximately 5.0 mg.100^{-1}g) by *Spirulina maxima* biomass. This can be explained due to copper antagonism or the inadequacy of the supplementation in the cultivation, where it presented with 17.3 % of zinc (Sm-Zn) and 82.7 % of inorganic salt. Sahin et al., (2013) also observed low zinc absorption during biosorption of this mineral in *Aspergillus tamarii* biomass (only 54.33 % of the 10 mg.L^{-1} used for the absorption). During the two weeks of experiments, Molnár et al., (2013) found values of 37.81 mg.100^{-1}g. Promising results were found in the present work, showing that the levels of magnesium and zinc incorporated in the microalga during six days of experiment can characterize it as an enriched compound, supporting food supplementation for people who have deficits in these minerals.

When comparing the results of magnesium and zinc concentration incorporated in the Spirulina biomass, together with the percentage of these minerals incorporated, an interesting phenomenon is observed. The highest percentages of magnesium incorporation are found in situations where its source is added in lower concentrations, regardless of the concentration of the zinc source present in the medium. This same phenomenon is observed in the percentage of incorporation of zinc, iron and selenium.

The present work deals with a system of incorporation of minerals in high cellular concentrations (5 $g.L^{-1}$), and as well as many organisms, Spirulina has regulatory mechanisms to prevent the imbalance of minerals in the normal metabolism of the cell. What can sometimes happen is that mineral elements compensate for the toxic effects of another mineral or simply replace the essential element in some less specific function, such as maintaining osmotic pressure (Buckley, 2019).

Among the possible mechanisms of mineral absorption, passive transport is one of them. This mechanism can be described according to Fick's Law, since the movement of molecules by diffusion may occur in favor of a concentration gradient until reaching equilibrium (Smith et al., 1999). In the present work, the phenomenon of diffusion through biological membranes is possibly a very restricted explanation, mainly due to the low permeability of the lipid bilayer for polar molecules.

Other possible explanations for the phenomenon of mineral absorption by Spirulina could include that described by Reid & Hayes (2003), where he describes the transport that occurs against the gradient of chemical or electrochemical potential and also the systems with transport proteins.

For iron incorporation, the highest value was obtained in experiment 10b (Table 4). This fact is justified by the use of the lowest level of sodium selenite concentration and the intermediate level of iron sulfate concentration.

Table 2. Actual and coded matrix of the Central Composite Design 2^2 used for the incorporation of iron and selenium in *Spirulina platensis* biomass. Exp: Experiment; IM: Incorporation medium; BM: Biomass.

Exp	$FeSO_4$ in the IM ($mg.L^{-1}$)	Na_2SO_3 in the IM ($mg.L^{-1}$)	Fe in BM ($mg.100^{-1}g$)	Se in BM ($mg.100^{-1}g$)
1b	(-1)54.57	(-1)36.5	42.75	1,141.55
2b	(+1)255.50	(-1)36.5	51.57	857.45
3b	(-1)54.57	(+1)163.5	39.99	1,215.07
4b	(+1)255.50	(+1)163.5	48.49	1,176.15
5b	(0)155.60	(0)100.0	67.61	1,738.81
6b	(0)155.60	(0)100.0	68.92	1,667.30
7b	(0)155.60	(0)100.0	69.50	1,557.15
8b	($-\alpha$)14.30	(0)100.0	12.78	1,158.59
9b	($+\alpha$)296.80	(0)100.0	59.68	1,542.83
10b	(0)155.60	($-\alpha$)10.2	73.50	1,278.80
11b	(0)155.60	($+\alpha$)189.8	56.93	1,560.66

Source: Authors.

A good repetition of the central points and the values close to them are found in experiment 10b. This can be explained by the fact that the best absorption is obtained from the concentrations close to the central points of iron. In the incorporation of 59.68 $mg.100^{-1}g$ (experiment 9b), it was observed that the highest level of iron concentration did not cause the greatest response. It is also verified that the incorporation of iron is strongly influenced by the iron sulphate heptahydrate concentration, as observed in experiment 8b. Saeid et al., (2013) found values between 14.3 e 18.0 $mg.100^{-1}g$ of Fe in

Spirulina maxima biomass. Molnár et al., (2013) found results of 78.9 mg.100^{-1} g during two weeks of experiments, showing that iron can be considered one of the limiting factors in *Chlorella* growth. Spirulina biomass growth was not significantly affected by the increased concentration of iron.

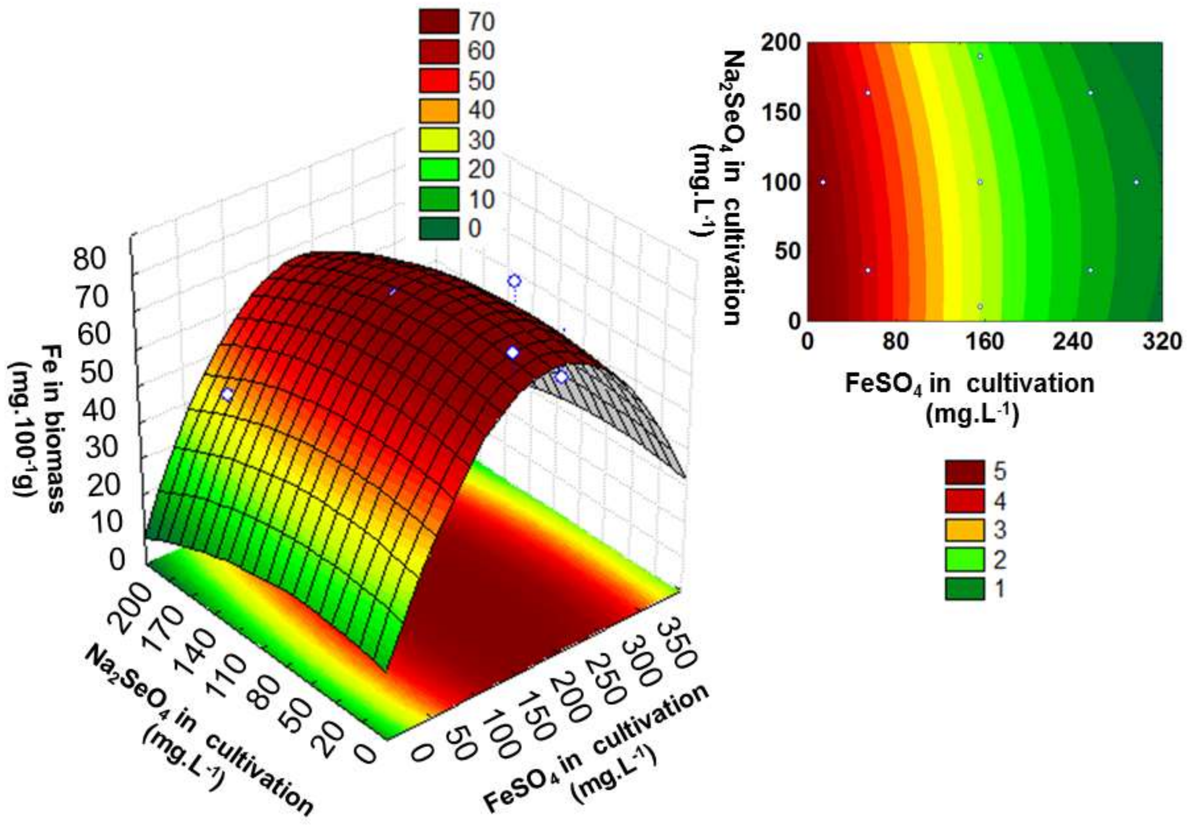
Iron bioavailability in the organic form shows an efficiency significantly superior to those originating from an inorganic form (Worms et al., 2006), which means a trend in the clinical use of these products for the treatment of iron deficiency anemia (Schümann et al., 2007). From the results obtained in Table 2, a multiple regression, obtaining a mathematical model capable of predicting the absorption of iron (mg.100^{-1}g) by *Spirulina platensis* biomass against variations of Iron sulphate and Sodium Selenite ($R^2 = 87.4$ %) was obtained, according to Equation 3.

$$[Fe] = 68.69 + 10.46 \times FeSO_4 \cdot 7H_2O - 17.56 \times (FeSO_4 \cdot 7H_2O)^2 - 3.66 \times Na_2SeO_3 - 2.98 \times (Na_2SeO_3)^2 - 0.08 \times FeSO_4 \cdot 7H_2O \times Na_2SeO_3 \tag{3}$$

Among trace elements, iron is one of the most essential elements required by microalgae. Iron, as a transition metal, is associated with enzymes through the formation of complexes with S or N groups of various amino acids. Iron is involved in fundamental enzymatic processes, such as oxygen metabolism, electron transfer, nitrogen assimilation and synthesis of DNA, RNA and chlorophyll (Naito et al., 2005). High concentrations of iron in some species of microalgae induce lipid synthesis and increase its content in biomass (Yeesang & Cheirsilp, 2011).

It was proven that only the iron sulphate concentration is significant at the level of 10 %, showing that the concentration of sodium selenite interferes little with the absorption of iron by *Spirulina platensis*. Figure 3 shows the response surface based on Equation 3 of the complete model. In Figure 3, it can be observed that the region with the highest experimental responses is close to the central region and the lowest responses are found in the lower levels of iron sulfate heptahydrate, according to the values obtained from experiments 1b, 3b and 8b.

Figure 3. Response surface of the incorporated iron concentration (a) and contour curves of the percentage of incorporated iron (b) in Spirulina platensis biomass.



Source: Authors.

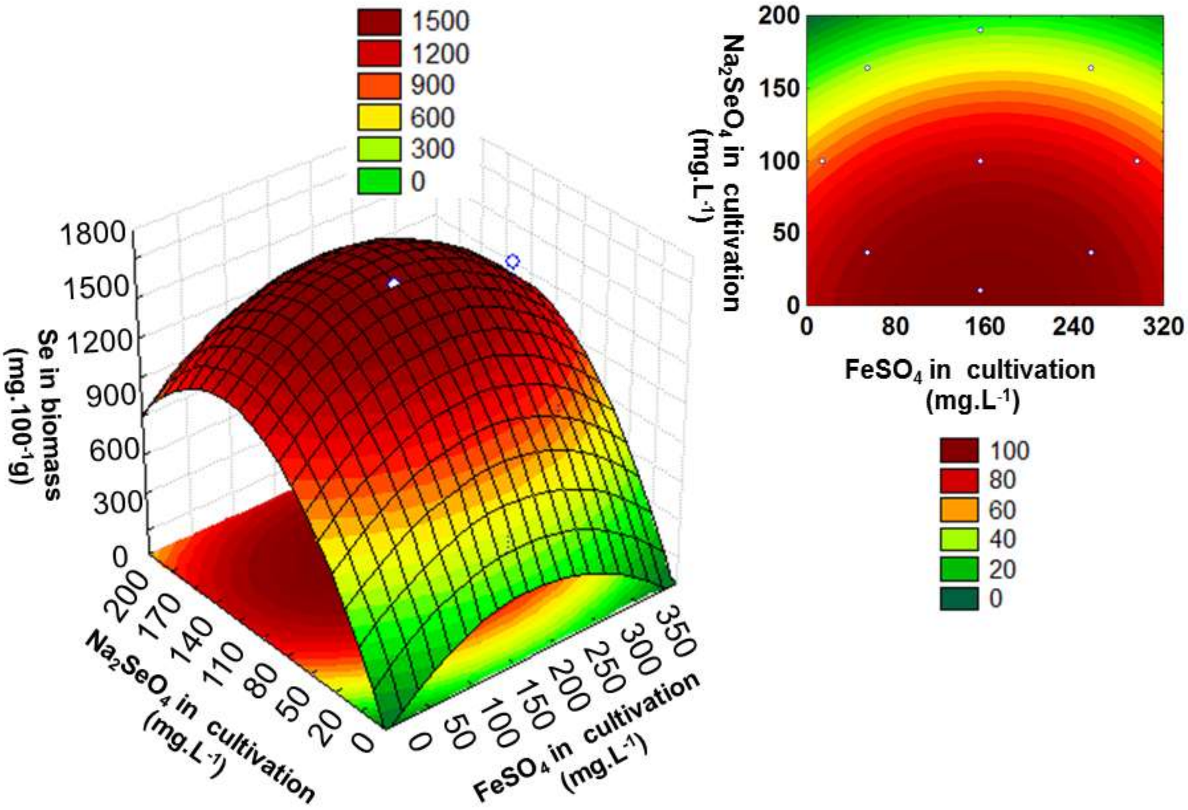
From the results obtained in Table 2, a multiple regression, obtaining a mathematical model ($R^2 = 63.3\%$), according to Equation 4 was obtained. This model is capable of predicting the absorption of selenium ($mg.100^{-1}g$) by *Spirulina platensis* biomass against variations of iron sulphate and sodium selenite.

$$[Fe] = 1{,}655.55 + 27.42 \times FeSO_4 \cdot 7H_2O - 225.27 \times (FeSO_4 \cdot 7H_2O)^2 + 99.00 \times Na_2SeO_3 - 190.55 \times (Na_2SeO_3)^2 + 61.29 \times FeSO_4 \cdot 7H_2O \times Na_2SeO_3$$

(4)

It was observed that the lowest response (857.45 $mg.100^{-1}g$) was obtained for experiment 2b with lower levels of sodium selenite and higher levels of iron sulfate. When compared to experiment 4b, it was observed that maintaining the same level for iron sulfate and practically quadrupling selenite concentration, led to a slightly increased response (1,176.5 $mg.100^{-1}g$, around 38 %). Figure 4 shows the response surface based on Equation 4 of the complete model.

Figure 4. Response surface of the incorporated selenium concentration (a) and contour curves of the percentage of incorporated selenium (b) in Spirulina platensis biomass.



Source: Authors.

Analyzing the response surface of Figure 4, it is observed that the region of greatest absorptions is around the central points.

Selenite is metabolized in chloroplasts by the same metabolic pathways as sulfur, due to their chemical similarity. This is a mechanism for selenium accumulation, after the formation of selenocysteine and subsequent bi-methylation to form dimethyl diselenide - DMDSe (Ellis & Salt, 2003).

Saeid et al. (2013) found a concentration of 0.125 mg.100^{-1}g of selenium in *Spirulina maxima* biomass, which is below the values found in the present study. During the increasing addition of Se in the cultivation medium to avoid the inhibition of *Spirulina platensis* growth, Chen et al., (2006) reached values of 1.033 mg.L^{-1}.

Tiantian et al., (2011) tabulated and confirmed maximum involvement of $–COOH$, followed by $–NH_2$, $–OH$, $–SO_3H$, and also $–P_2O_3$ groups, to generalize the main functional groups involved in metal binding in microalgae. Léon et al., (2007) mentioned the role of sulfate in the glycoproteins of the cell wall in metal binding and elaborated the role of carboxylate and sulfate groups involved in pH-dependent binding of cadmium. Kotrba et al., (2011) specified the carboxyl-, hydroxyl-, sulfate and amino groups in the algal cell wall polysaccharides, acting as binding sites for metals. They also confirmed that functional groups containing O-, N-, S-, or P-, participate directly in the binding of certain metals. In addition, the contribution of other functional groups in the cells and in the cell walls of algae and fungi, such as the strongly acidic sulfate groups $R–OSO_3$ has also been reported.

Evaluating the results of absorption of minerals in the present work, we found that in the absorption processes by Spirulina, the mineral that is most easily absorbed was selenium (1738.81 mg.100^{-1}g, representing 86.94 % of all selenium added), followed by magnesium (410 mg.100^{-1}g, representing 1.03 % of all magnesium added). However, the absorption of iron and zinc, despite reaching important concentrations in biomass, represented the lowest absorption rates by Spirulina, being 68.92 mg.100^{-1}g (representing 2.21 % of all iron added) for iron and 34.84 mg.100^{-1}g (representing 8.71 % of all zinc added) for zinc.

Another possible explanation for the high results of the incorporation of selenium and magnesium may have been due to the transport mediated by carriers, considered as passive transport and sometimes known as facilitated diffusion of these minerals in particular. Although this phenomenon is similar to diffusion, transport occurs due to a gradient, that is, diffusion occurs as a result of a concentration gradient and passive transport via carrier occurs due an electrochemical potential gradient (Lodish et al., 2000).

Certainly, the phenomenon of simple diffusion did not predominate, since the rate of transport into the cell was not proportional to the external concentration of the transported molecule. The result of this phenomenon is a completely passive and consequently very slow transport (Newman, 2001). This statement is more easily observed for the magnesium incorporation, since the concentration of this mineral in the incorporation medium was much higher than the other minerals.

On the other hand, carrier-mediated transport tends towards a maximum rate, which is achieved when all the substrate-binding sites are occupied. The carrier concentration makes the rate limiting, that is, the concentration of the mineral capable of reaching half the maximum absorption speed, tends to reflect on the properties of the binding site, on its specificity and selectivity (Reid & Hayes, 2003).

Biosorption of metals by microalgae is generally a two-phase process (Das et al., 2008). The first phase is the adsorption by extracellular materials associated with cells, for example, polysaccharides and mucilage (De Philippis et al., 2011) and cell wall components, for example, carboxy and hydroxy groups, as well as sulfate and phosphate (Naja & Volesky, 2011). This is a fast, non-metabolic process, occurring in living and non-living cells and it is dependent on pH, metal species, biomass concentration (Naja & Volesky, 2011) and type of seaweed (De Philippis et al., 2011).

The second phase is absorption and accumulation within the cell. This is a process that involves active transport across the cell membrane and attaches to proteins and other intracellular components. It is a metabolism-dependent mechanism and occurs only in living cells (Richmond & Hu, 2013).

Despite the difficulty in demonstrating the mechanisms involved in the absorption of metals and minerals in microalgae biomass, the present work presents promising results regarding the incorporation of high concentrations of magnesium, zinc, iron and selenium in *Spirulina platensis* biomass. Such results can guarantee a bio-enriched biomass, with the possibility of a significant increase in the absorption of these minerals in human or animal feed. This can guarantee the use of this biomass as raw material for the development of foods with metabolic and physiological effects, acting in the growth, development, maintenance and improvement of diverse functions of the human and animal organism.

4. Conclusions

According to the results found in the present study, it can be observed that high concentrations of Mg, Zn, Fe and Se did not significantly influence *Spirulina platensis* growth during the 6-day process. The concentration of magnesium sulfate acts in increasing magnesium absorption by *Spirulina platensis* and practically does not interfere with the incorporation of zinc. However, the zinc sulfate concentration does not interfere with the absorption of this mineral. Similar behavior is observed regarding the concentration of iron sulphate heptahydrate since the incorporation of iron is strongly influenced. The best selenium biosorption was observed in the experiments with lower concentration of sodium selenite and higher concentration of iron sulfate. The most absorbed mineral was selenium, followed by magnesium, zinc and iron, showing that its absorption occurs possibly, from carriers and facilitated diffusion. The biomass obtained can be characterized as a rich compound in Mg, Zn, Fe and Se. Such results suggest the continuity of the research in the development of foods and evaluation of the absorption in individuals that present some deficiency of the minerals incorporated in the Spirulina biomass.

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