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Research Paper

A study of bio-hybrid silsesquioxane/yeast: Biosorption and neuronal toxicity of lead

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ABSTRACT

Lead is a heavy metal of high impact for the environment as well as for human health, being cause of several diseases. Considering the importance of obtaining an effective treatment for lead removal, a new hybrid material was developed for sorption of Pb^{2+} from aqueous solution. The effect of pH, temperature, liquid/solid ratio (g/cm^3) and lead concentration on the sorption capacity of yeasts chemically modified with cubic silsesquioxane (YS) was analyzed. Additionally, the toxicity of lead on the neuronal activity was also investigated in order to assess whether the damage caused by the Pb^{2+} ion is reversible or not. The YS is highly promissory as sorbent of lead in high concentrations (100 and 500 ppm), reaching high efficiency in short contact times (15 min), and at the natural pH (4) of the Pb^{2+} solution and room temperature. The best sorption obtained was 82% removal and 248 mg/g with 500 cm^3/g sorbent, pH 4, room temperature and contact time of 15 min. Besides, such high efficiencies are obtained with low quantities of biosorbent, when compared with other similar materials. The impact of lead on neuronal function was studied by measuring autofluorescence signals, associated with changes in cellular metabolism, at the hippocampal CA3 area in brain slices. In this toxicity tests, the effect of low concentrations of lead (1 and 3 μM) on neuronal activity was evaluated. After removal of the lead, the irreversibility of the observed changes can be verified, which suggests the existence of neuronal damages.

1. Introduction

The release of drugs and heavy metals into the environment and their effects on living organisms has been reported as one of the 40 more important conservation and management policy priorities established by scientists around the world (Fleishman et al., 2011). Some results report that these contaminants may cause diseases related to the nervous system, birth defects, and some cancer types (Birkett and Lester, 2003; Colborn et al., 2002; Birnbaum and Fenton, 2003).

Among the disruptors from inorganic origin, it was found that heavy metals do not degrade and eventually may reach living beings (Birnbaum and Fenton, 2003; Wang and Chen, 2006). Indeed, sometimes, industrial activity converts metals into chemical forms within products that can be more toxic to man. Lead (Pb), for example, is an important metal with many industrial uses. Its widespread industrial application leads to its discard in surroundings and consequently to human exposure through food sources and the presence in drinking water (Goyer, 1990).

Vertebrates contaminated with metals such as lead, exhibit dysfunctions in the nervous system, bone marrow, kidneys, and in fetal development due to their ability to traverse the placenta (Moreira and Moreira, 2004). The use of brain slices is one way to evaluate the damage on human health, because metals like lead act directly on the central nervous system inducing acute encephalopathy or changes in behavior and cognition. Cerebrovascular damage may also occur for acute high doses (Clarkson, 1987).

Specifically, lead accumulates in the hippocampus and amygdala during animal development, reducing the dimension of the first brain structure (Audesirk, 1985). Metals, including lead, affect various pre- and postsynaptic functions and synaptic plasticity, having a wide range of cellular consequences (Sadiq et al., 2012). Lead, in particular, may interfere simultaneously with several ionic channels and receptor

Abbreviations: CSS, cubic silsesquioxanes; YS, yeast modified with silsesquioxane; POSS, polyhedron oligosilsesquioxane; SS, silsesquioxane

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mechanisms, with transmitter binding and uptake and in the expression of long-term plasticity. Thus, it likely interferes with autofluorescence responses implicated in mitochondrial activity, associated with neurotransmission (Kosterin et al., 2005; Shuttleworth, 2010). For these reasons, the effect of lead on neuronal autofluorescence was studied at the zinc-rich hippocampal mossy fiber synapses that are characterized by special long-term plasticity (Nicoll and Schmitz, 2005; Quinta-Ferreira et al., 2004). Flavoproteins autofluorescence, mainly with mitochondrial origin, was detected using visible excitation light, responding NADH to ultraviolet light (Kosterin et al., 2005; Shuttleworth, 2010).

Nanotechnology, an emerging technology, is prone to be applied in the effort to reduce costs and improve the overall effectiveness of the methods of environmental remediation (Karn et al., 2009; Zeng et al., 2017; Hu et al., 2016a), and has important biomedical applications (Kannan et al., 2005) as well. In this context, silsesquioxanes an important class of nanostructured materials has attracted huge interest in different areas of knowledge. The silsesquioxanes are compounds that have the empirical formula $(RSiO\ 1{,}5)_n$, where R can be a hydrogen or an organic group methyl, vinyl, phenyl, alkyl, alkylene, aryl, arylene or organofunctional derivatives thereof (Baney et al., 1995; Lim et al., 2012), n is an even number ($n \geq 4$) which can vary, being the resulting compound named polyhedron oligosilsesquioxane (POSS) or polysilsesquioxane when n is an undefined number (Voronkov and Lavrentyev, 1982; do Carmo et al., 2010). The reactivity and functionality of the compound can be changed by modifying the functional group R with a variety of organic and inorganic substituents (Baney et al., 1995; Voronkov and Lavrentyev, 1982). There are numerous applications of this compound when used as precursor in the formation of organic and inorganic hybrid compounds (do Carmo et al., 2010; do Carmo et al., 2008; Hu et al., 2016b), in catalysis (Wada et al., 2009), as additive (Bourbigot et al., 2009), polymer (Soh et al., 2007), liquid crystal (Mehl and Goodby, 1996), in electroactive films and compounds (Lucho et al., 2004; do Carmo et al., 2015; da Silveira et al., 2013), as precursor of dendrimers (do Carmo et al., 2007), in targeted delivery of therapeutic genes (Kaneshiro et al., 2007) among others.

In this work, silsesquioxane was chemically modified by adding yeast (*S. cerevisiae*). This is a eukaryotic cell with a completely sequenced genome (Goffeau et al., 1996), that can be easily manipulated and is often applied in industrial systems that use fermentation and biosorption processes (Kapoor and Viraraghavan, 1995; Wu et al., 2012; Zhang et al., 2009a). In fact, yeasts have been extensively used for biotechnological purposes, such as bioremediation of effluents contaminated with metals and organic compounds, and for the generation of bioenergy, because of their great performance in fermentation processes (for review see (Ostergaard et al., 2000)). Also, various biomedical applications including actions on the immune system, adsorption of toxic substances, nutrient enrichment and carrier of bioactive molecules (Jenabian et al., 2010) are pointed out as relevant applications. Indeed, yeasts have great metabolic diversity, being able to use different sources of carbon and nitrogen, resist to wide changes in osmotic concentration and pH, from alkaline to pH 2.5, (Lujan et al., 1994; Drake and Rayson, 1996; Smith et al., 2001; Kordialik-Bogacka, 2011) and are able to grow at temperatures up to 50 °C (Abdel-Banat et al., 2010). Furthermore, the composition of the cell wall of yeasts, formed mainly by glucans, mannans, chitin, proteins and lipids (Tortora et al., 2005), has great potential to be used as biosorbents: acetamide groups of chitin, structural polysaccharides of fungi, amine groups and phosphate in nucleic acids, amine, amide, sulfhydryl, carboxylate in protein and polysaccharide hydroxyl groups are chemical groups that can sequester and pre-concentrate the metal (Volesky, 2007).

The chemical modification of yeast with the silsesquioxanes targets to increase its ability towards biosorption of metals and organic analytes with biological and environmental interest (organic disruptors). The cubic silsesquioxane has to be used in its eight vertices peripheral $-NH_2$ groups, which are susceptible to enter in chemical reactions of nucleophilic substitution, which can occur on the surface (walls) of the yeasts. In this regard, this material intends to chemically modify the walls of yeast by exploiting the reactivity of the nanostructures presented by silsesquioxanes.

Thus, these studies aim to provide information about the potential involved in the lead biosorption process in living cells by the modified yeast. The intention is to enhance the adsorption of lead and apply it as a possible remediation of contaminated water streams.

2. Experimental

2.1. Synthesis of silsesquioxane $Cl[H_3N(CH_2)_3]_8Si_8O_{12}$

The silsesquioxane $Cl[H_3N(CH_2)_3]_8Si_8O_{12}$ (SS) was prepared according to the method proposed in the literature (Feher et al., 1999): 3600 mL of methanol, 200 mL of hydrochloric acid (HCl) and 150 mL of γ- aminopropyltriethoxysilane were added into a round bottom flask of 6000 mL. The system was kept under constant stirring at room temperature for 6 weeks. The product obtained (SS) was washed using thin sintered plate funnel with cold methanol, and the product was dried at 100 °C.

2.2. Yeast modified with silsesquioxanes (YS)

5.0 g of SS are added to 100 mL of deionized water followed by the addition of 11.57 g of sodium bicarbonate ($NaHCO_3$) (Magossi, 2015). After a stirring period, 5 g of dry yeast are added and the mixture is left under rigorous stirring for further 12 h. Then the suspension is centrifuged and the separated solid phase is washed thoroughly with deionized water. The final product will be described as YS (yeast modified with silsesquioxanes).

2.3. Biosorbent

Five biosorbents were applied in these tests: yeast *Saccharomyces cerevisiae* Type II (Sigma-Aldrich)(YST), Silsesquioxane (SS), yeast *Saccharomyces cerevisiae* Type II (Sigma-Aldrich) modified with Silsesquioxane (YS), dry yeast *Saccharomyces cerevisiae* encapsulated from a winery – ProElif® – (YW) and yeast *Saccharomyces cerevisiae* encapsulated from a winery after fermentation process – ProElif® – (YWF). YW and YWF were used for comparison purposes, since these materials are used in an industrial process, especially YWF that is a waste of the alcoholic fermentation process and can thus be of low cost. Both were obtained in Adega Cooperativa de Cantanhede (Cantanhede-Portugal).

2.4. Biosorption

The sorption tests were carried out with $Pb(NO_3)_2$ solutions with different Pb^{2+} concentrations: 100, 500 and 1000 mg/L. The suspensions (25 mL) were shacked at 90 rpm, centrifuged (Nahita 2650) during 2 min at 4000 rpm and the supernatants were collected.

The effect of the biosorbent load was assessed for several concentrations of the dry biosorbents: 50, 100, 250 and 500 cm^3/g. The effect of pH on biosorption was investigated by adjusting and maintaining pH of the effluent at pH values of 4, 5, 6 and 7. For pH adjustment, 0.1 M HCl and 0,1 M NaOH solutions were used. The tests for evaluating the effect of temperature were performed at 25 and 40 °C.

The concentration of Pb^{2+} in solution was measured by atomic absorption spectrophotometry (ContrAA 300 – Analytikjena) at 283.31 nm (Baroni et al., 2017; NIOSH, 1994).

2.5. Toxicity of lead

Toxicity tests were made in hippocampal slices (400 μm thick), from female Wistar rats (12 weeks old). The slices, obtained from animals

sacrificed by cervical dislocation, were submerged in oxygenated (95% O_2, 5% CO_2) artificial cerebrospinal fluid (ACSF), for at least 1 h. After this period the slices could be transferred to the recording chamber, and perfused (at 1.5 mL/min) with oxygenated ACSF. The recording chamber is inserted in a setup containing a fluorescence microscope (Zeiss – Axioskop) equipped with a water immersion lens (40x, N.A. 0.75, 1.6 mm working distance). The optical signals were recorded in the hippocampal CA3 area, at the mossy fiber-CA3 pyramidal cells synaptic system, using a tungsten/halogen lamp (12 V, 100 W) and excitation and emission wavelengths of 480 nm (10 nm bandwidth) and above 500 nm, respectively. The signals were detected using a photodiode (Hammamatsu K2G 1336). After passing through an I/V converter with a 1 GΩ feedback resistance, they were applied into an AC-coupled amplifier with a low (1 Hz) cut-off frequency and processed through a data acquisition system from National Instruments™, using the Signal Express analysis software. The points in the graphs, represented at 2 min intervals, are the mean of 2 consecutive data points, having each been obtained as the average of 100 sequentially recorded data. The extracellular ACSF medium had the following composition (in mM): NaCl 124.0; KCl 3.5; $NaHCO_3$ 24.0; NaH_2PO_4 1.25; $MgCl_2$ 2.0; $CaCl_2$ 2.0 e D-Glucose 10.0, pH 7.4, 32° C. The lead solutions were prepared from a 1 mM $PbCl_2$ stock medium. For the tests, concentrations of 0.2 and 0.6 mg/L $PbCl_2$, equivalent to 1 µM and 3 µM, respectively, were used. All experiments were carried out in accordance with the European Communities Council Directive. All efforts were made to minimize animal suffering and to use only the number of animals necessary to produce reliable scientific data.

The chemicals used were: $PbCl_2$; NaCl, KCl, $NaHCO_3$, NaH_2PO_4, $CaCl_2$, $MgCl_2$, D-glucose (Sigma, Sintra, Portugal).

3. Results and discussion

3.1. Lead biosorption

Firstly, the material synthetized used in the sorption test was characterized by Fourier-transform infrared spectroscopy (FTIR) in order to confirm the modification of the material. The results are showed in Fig. 1.

FTIR spectrum of silsesquioxane (Fig. 1A) displays different absorption bands suggesting the complex nature of the material. Peaks identified at 3428 cm^{-1} and 3137 cm^{-1} correspond to the N—H stretching vibration (νN—H). The peak observed at 2903 cm^{-1} is due to C—H (νC—H) deformation, while the ones identified at 1610 e 1583 cm^{-1} may be assigned to the deformation N—H(δN—H). The peak at 1106 cm^{-1} may be assigned to symmetrical axial stretching (Si—O—Si) (ν Si—O—Si) and at 706 cm^{-1} corresponds to the Si—C (νSi—C) stretching related to the Si—C bond in $SiCH_2$ (Magossi, 2015; Feher and Wyndham, 1998). Regarding the yeast spectrum (Fig. 1B), it is visible the occurrence of absorption bands between 3030 and 2800 cm^{-1}, and 1500 and 1350 cm^{-1} that characteristics of the lipid acyl chains. The peaks at 1740 cm^{-1} and 1500 cm^{-1} should be attributed to the C═O stretching of esters and N—H deformation (δN—H) of peptide bonds, respectively. The band between 1250 and 1000 cm^{-1} is due to the contribution of phosphodiester groups of nucleic acids and phospholipids, in addition to C—O uptake of carbohydrates (Ami et al., 2014). For the modified material (Fig. 1C), it was found that its spectrum exhibits bands identified in the previous materials. Therefore, we have the following bands: 3030–2800 cm^{-1} of the yeast acyl chains; 1740 cm^{-1} for the C═O stretching of yeast esters; 1500 cm^{-1} of the N—H deformation (δN—H) of the peptide bonds also of the yeast; 1106 cm^{-1} of symmetrical axial stretching (Si—O—Si) (ν Si—O—Si) and 706 cm^{-1} for the Si—C (νSi—C) stretching, both of typical of the silsesquioxane. Thus, it is possible to verify the connection between the materials, attesting the chemical modification and respective formation of the new compound.

The sorption capacity of the different materials was measured from a solution with 100 ppm of lead initially.

The values found in the literature referred in Table 1, where the maximum Pb^{2+} removal for various biosorbents materials with different initial lead concentrations, pH and temperature are displayed, were used as a reference to evaluate the results obtained in this study.

Our results depicted in Fig. 2 reveal that the functionalized yeast with silsesquioxane, YS, presents the maximum sorption potential with 100% of lead removal what corresponds to a sorption capacity of 102 mg/g. Indeed, this new material is markedly better sorbent than YST-basic yeast – (44% removal, 49.4 mg/g), which is followed by dry yeast *Saccharomyces cerevisiae* encapsulated from winery ProElif® (YW) – (37% removal, 17.8 mg/g), yeast *Saccharomyces cerevisiae* encapsulated from winery after fermentation process ProElif® (YWF) (25% removal, 12.6 mg/g) and SS – octa-(3-cloropropil)silsesquioxane – (1% removal). This test was performed with 25 mL of lead solution (100 ppm), 15 min of contact time, 25 °C and 90 rpm agitation.

In this context, two materials were then selected, YS and YST, for comparison. The YST was chosen because it is the base material to YS. Then, the comparison between the original and modified material is indicated. A more detailed analysis of the behavior of the modified Yeast with silsesquioxane was then further assessed by studying the effect of various parameters on its removal capacity, including the concentration of lead, pH, temperature and sorbent concentration, aiming to reach optimal conditions for LS biosorption.

In Fig. 3, a wide range of the metal load was tested by using different initial concentrations of ion lead (100, 500 and 1000 ppm). The concentrations of Pb^{2+} tested in this work were based on the existence of sites with lead contamination at levels varying in the range 300–1000 ppm (Machado et al., 2013). It is important to look for high performance materials at high concentrations. As observed, the functionalized yeast, YS, removed 100% (102 mg/g) of lead, for initial Pb^{2+} 100 ppm, 82%, (248.04 mg/g) with lead 500 ppm and decreased to 22% (70.83 mg/g) when Pb^{2+} was 1000 ppm. For concentrations higher than 100 ppm, the highest sorption capacity observed in Table 1 was 144 mg/g at 200 ppm of lead, but these results were obtained with the double amount of sorbent (1000 cm^3/g). Therefore, the reference uses more sorbent for less metal sorption in comparison with this work where only 500 cm^3/g of sorbent were used.

The contact time of 15 min was considered in all the equilibrium experiments based on preliminary tests where the 100 ppm sorption of lead was maintained at 100% with YS in a time range of 5 min–2 h (Data not shown).

The YST sorbent just removed 27% (27.7 mg/g), of lead in 100 ppm solutions, 13% (39.8 mg/g) in 500 ppm and 0% in 1000 ppm. These results reveal that the sorption capacity increases initially, and decreased with the increase of the metal concentration to 1000 ppm, in both tests

El-Sayed (2013) showed that the sorption capacity increases with increase lead concentration but in concentration higher than 300 ppm, the sorption capacity decreases. This occurs due to the saturation of the bindings sites. In this work, the increase of sorption capacity between 100 and 500 ppm followed by a reduction when initial Pb^{2+} concentration reaches 1000 ppm shows the saturation of the materials.

Since YS removed all lead in the solution of 100 ppm, the initial Pb concentration that was chosen for the subsequent tests, involving the effect of pH, temperature and sorbent concentration, was 500 ppm.

The pH influence on the sorption capacity is showed in Fig. 4, where it is observed that the ideal value for YS is pH 4, which is the original solution pH. In this case, the metal binding leads to a sorption capacity of 248.04 mg/g with 82% of lead removal, and the lower value is observed at pH 6 (133.7 mg/g) with 48% of lead removal. For YST the optimum pH is around 4, where the sorption capacity is of 39.8 mg/g (13%). The literature results analyzed in Table 1 show that most of experiments are performed at pH 5. pH values higher than pH 6 were not tested in our case because the Pb^{2+} ions precipitate above this pH.

The pH values of 4, 5 and 6 were tested because are reported as

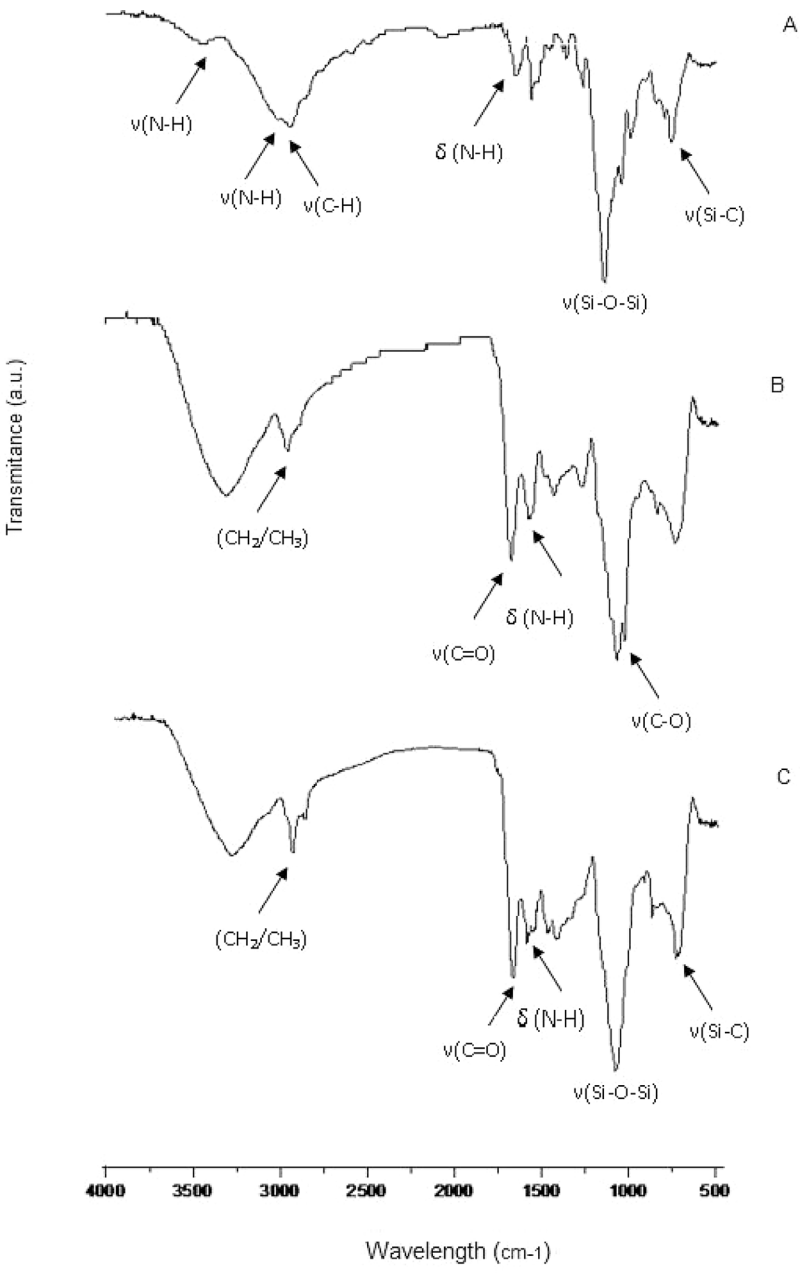

Fig. 1. FT-IR spectra of: (A) SS (Silsesquioxane), (B) YST (Yeast) and (C) YS (Yeast modified with SS).

Table 1
Literature review about biosorption of lead.

Maximum metal-binding (mg/g)	Biosorbent Material	Lead Concentration (ppm)	pH	Liquid-Solid ratio (cm^3/g)	Temperature (°C)	Contact Time	Reference
72.5	Yeast suspension	10–108	5	~650	22	8 weeks	Amirnia et al. (2015)
160	Biomass from biotrickling filters	50	5	2000	25	210 min	Cheng et al. (2015)
2.34	Yeast from fermentor brewery's	102	5	50	25	120 min	Parvathi et al. (2007)
6.52	Yeast from brewery	40	–	312	25	> 180 min	Zhang et al. (2009b)
144	Yeast culture dried	200	5	1000	25	1440 min	Özer and Özer (2003)
85.57	Brewery's waste biomass	207	4	500	30	180 min	Chen and Wang (2008)
51.81	Brewer's yeast	53.75	4	400	25	60 min	Yahiaoui et al. (2011)
5.74	Waste beer yeast	101	5	125	20	30 min	Han et al. (2006)

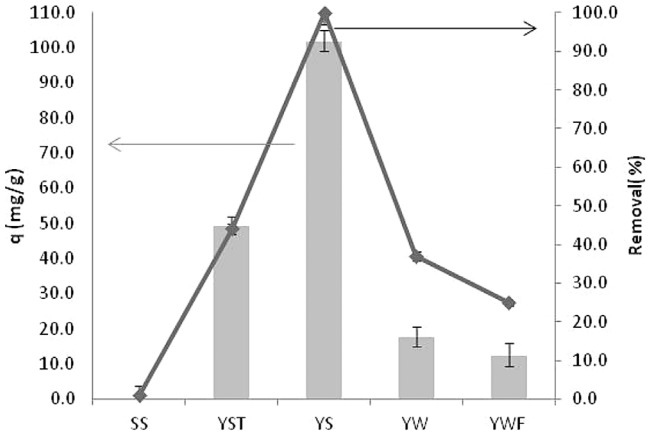

Fig. 2. Sorption capacity (q (mg/g)) and removal percentage (%) of lead with different materials: SS, YST (Yeast), YS (Yeast modified with SS), YW (yeast encapsulated from winery), YWF (yeast encapsulated from winery after fermentation). Initial lead solution: 100 ppm; Biosorbent: 500 cm^3/g; pH 4; Contact time: 15 min.

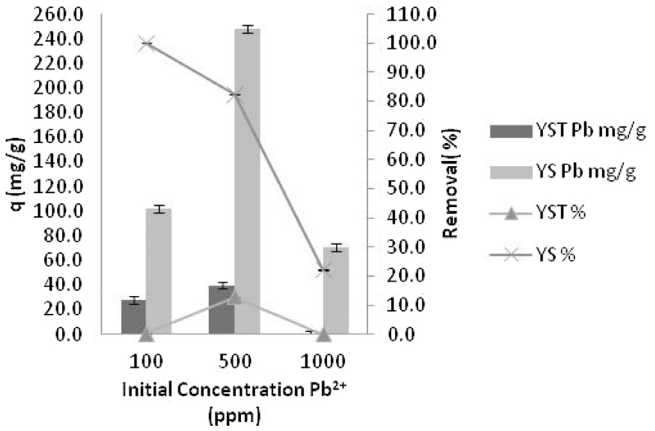

Fig. 3. Sorption of lead using different initial concentrations of Pb^{2+} with YST (Yeast) and YS (Yeast modified with SS). Initial Biosorbent: 500 cm^3/g; pH 4; Contact time: 15 min.

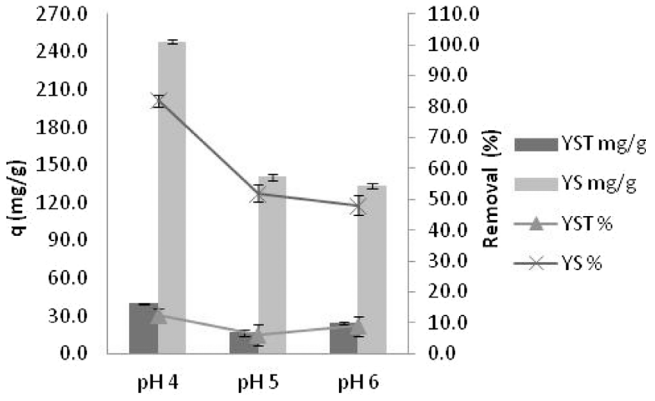

Fig. 4. Sorption of lead at different pH with YST (Yeast) and YS (Yeast modified with SS). Initial lead solution: 500 ppm; Biosorbent: 500 cm^3/g; Contact time: 15 min.

favorable to biosorption of metals. Vijayaraghavan and Yun (2008) describe that pH 3 and 6 are the most indicated to this process because of the deprotonation of carboxyl groups, where the greatest part of the metal binds by ion exchange.

The temperature effect is represented in Fig. 5. The temperature range was selected based on the work of Han et al.(2006) which shows that at temperatures higher than 25 °C the lead sorption capacity is reduced. Furthermore, the increase in temperature modifies the

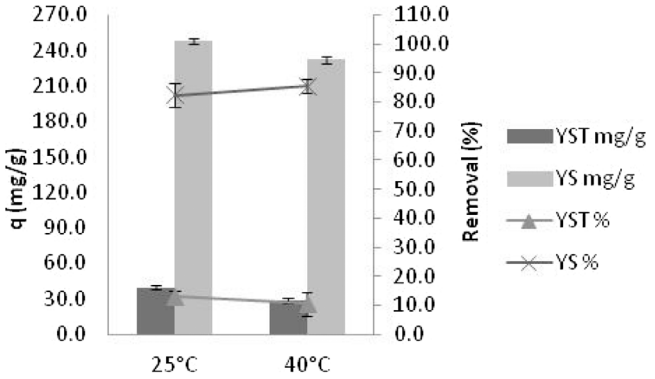

Fig. 5. Sorption of lead for different temperatures with YST (Yeast) and YS (Yeast modified with SS). Initial lead solution: 500 ppm; Biosorbent: 500 cm^3/g; pH 4; Contact time: 15 min.

sorption sites of the yeast by changing their interactions and sorption capacity (Labuto et al., 2015). For the YS in 40 °C, the biosorption is higher than that observed at room temperature (25 °C), even though the difference is only 3%. In YST, the temperature elevation reduced in 2% the removal percentage. Therefore, these results show that the variation of temperature does not strongly affects sorption. Thus, taking into account the cost of heating, the application of room temperature is more viable.

Assuming 500 ppm of lead in the initial solution, pH 4 and 25 °C, the influence of the sorbent concentration in the sorption capacity is analyzed in Fig. 6, where it is showed that the increase of the sorbent mass (decrease of the liquid/solid – L/S – ratio), increases the percentage removal and decreases the sorption capacity (mg/g): more adsorbent, more sorption, minor metal-binding per gram of adsorbent. In the YS case, for the liquid/solid (cm^3/g) ratios of 50, 100 and 250, the percentage removal of lead was 99%, with 50 cm^3/g, and 100% with 100 and 250 cm^3/g (27.5 mg/g, 56.4 mg/g and 143.2 mg/g, respectively) but the best sorption capacity is with 500 cm^3/g, where the weight of LS was 0.05 g in 25 mL of lead solution, and the sorption capacity was 248 mg/g, but lower percentage removal – 82%. This occurs because, for higher L/S values, lower mass of sorbent is applied so that the amount of lead per gram of sorbent is higher.

For the YST sorbent, the lead removal percentage was 48, 30, 27 and 13% with the liquid/solid ratios of 50, 100, 250 and 500 cm^3/g, respectively, while regarding the sorption capacity: 13.1, 16.4, 22.4 and 39.8 mg/g were reached. These results show a similar behavior when compared with YS sorbent: the removal percentage decreases and the sorption capacity (mg/g) increases by rising cm^3/g. In Table 1 the two

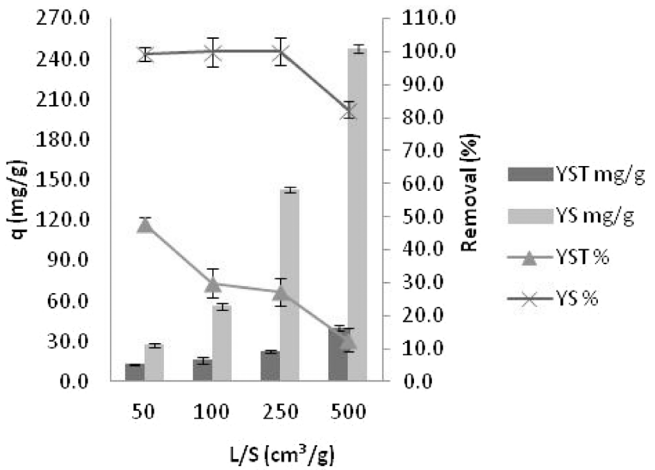

Fig. 6. Sorption of lead for different liquid/solid (cm^3/g) ratios with Lev (yeast) and LS (Yeast modified with SS). Initial lead solution: 500 ppm; pH 4; Contact time: 15 min.

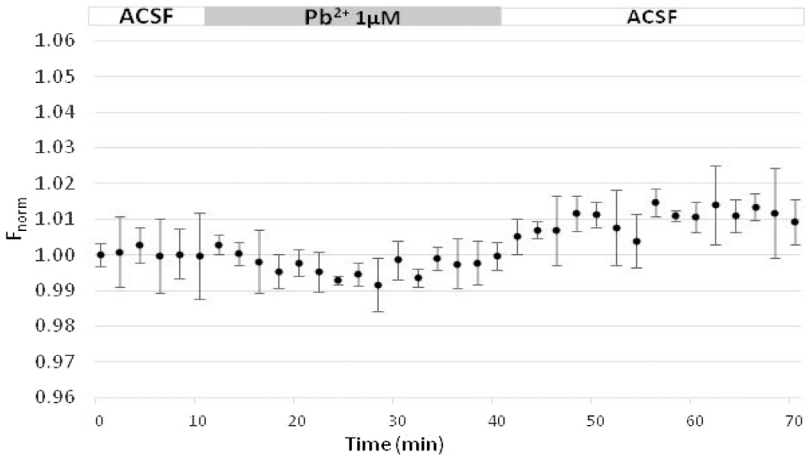

Fig. 7. Autofluorescence signals from brain slices exposed to 1 µM $PbCl_2$. After 10 min in ACSF the lead solution (1 µM) was applied for 30 min, followed by an equal period in ACSF, as indicated by the bars. The data were normalized by the average of the first 10 data points. All points represent the mean ± SEM (n = 3).

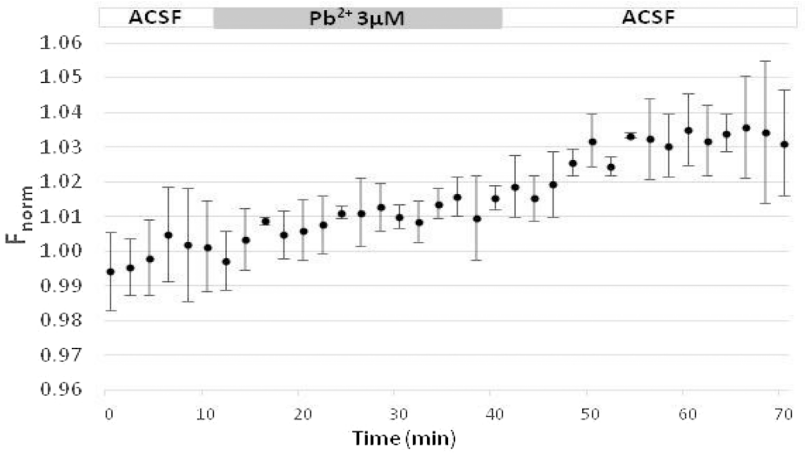

Fig. 8. Effect of 3 µM $PbCl_2$ on autofluorescence changes from brain slices. These were sequentially perfused by ACSF (10 min), $PbCl_2$ (3 µM, 30 min) and ACSF again (30 min). The normalized data points represent the mean ± SEM (n = 3).

higher values of sorption capacity (160 and 144 mg/g) were obtained with more than 500 cm^3/g (2000 and 1000 cm^3/g, respectively) what shows the efficiency of yeast in metal binding with less sorbent mass.

This way, for the new bio-sorbent material, YS, the best liquid/solid ratio is 250 cm^3/g, where the metal binding is complete (100%) in a solution with 500 ppm of lead. The use of 500 cm^3/g is indicated in lower lead concentrations because, in this ratio, the sorption of lead is 100%.

Finally, the new synthetized material (YS) has the greatest sorption capacity of lead in comparison with the results of the literature, where the yeast dried in 200 ppm of lead, and 1000 cm^3/g (double of the load used in this work), showed the sorption capacity of 144 mg/g in 1440 min (Özer and Özer, 2003). A similar material, from biotrickling filters, has the sorption capacity of 160 mg/g using 50 ppm with 2000 cm^3/g in 210 (Cheng et al., 2015). The YS that showed fast sorption of lead (15 min) with reduced liquid/solid ratio (500 cm^3/g), obtained 248 mg/g of sorption capacity in 500 ppm of lead.

The lower time used in literature of Table 1 was 30 min for yeast from waste beer, in 100 ppm of lead, obtained 5.74 mg/g of sorption, approximately forty times lower than the value in this work in 15 min of contact (Han et al., 2006).

3.2. Lead neurotoxicity

The effect of lead in neuronal activity was evaluated in brain slices, at the hippocampal mossy fiber synaptic region from CA3 area. Autofluorescence studies, evoked by different concentrations of $PbCl_2$ (1 and 3 µM), were performed using visible excitation light (480 nm) to detect flavoproteins fluorescence changes. In the presence of 1 µM (0.2 ppm) $PbCl_2$, applied for 30 min, the autofluorescence signals had a mild transient decrease, reaching again the baseline. After washout, there was a small increase towards a steady level, that was 1.01 ± 0.8% above baseline, in the period 60–70 min, as shown in Fig. 7. The effect of Pb^{2+} is not reversible, since the signal remains potentiated upon returning to the normal extracellular medium (ACSF).

In Fig. 8 the lead concentration was 3 µM (0.6 ppm) and the autofluorescence increased always during the lead application, being 1.01 ± 0.7% (n = 3) higher than baseline, during the interval 30–40 min. The signal continued to increase following washout reaching, in the last 10 min, a stable value that is 1.03 ± 2% above control.

It has been shown, in cultured hippocampal neurons, that even nanomolar concentrations of lead block the release of neurotransmitters (Braga et al., 1999). Another work, performed in the same neuronal preparation, associated lead damage with the formation of reactive oxygen species (ROS). Yin et al. (2008) found in their studies, using a much higher lead concentration (20 µM Pb^{2+}), a decreased neuronal viability and an increase in ROS formation that was irreversible.

Our work confirms the irreversibility of the lead effect, being the enhancement observed upon washout likely associated with a decrease in mitochondrial activity caused by lower cellular energy requirements. An opposite autofluorescence effect, evoked by electrical stimulation, was observed in cerebellar slices (Jotty et al., 2015).

4. Conclusions

The new synthesized material, yeast modified with silsesquioxane, is highly promissory as sorbent of lead in high concentrations (100 and

500 ppm), reaching high efficiency in short contact times (15 min) and at the natural pH (4) of the Pb^{2+} solution and room temperature. The best sorption obtained was 82% removal and 248 mg/g with 500 cm^3/g sorbent, pH 4, room temperature and contact time of 15 min. Besides, such high efficiencies are obtained with low quantities of biosorbent, when compared with other similar materials. In the neuronal studies, it was observed that low lead concentrations (1 and 3 μM) affected synaptic activity. The detected changes reveal the existence of a potentiation of the autofluorescence signals, following lead washout that may be associated with neuronal damage.

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