Desalination and Water Treatment
www.deswater.com

doi: 10.1080/19443994.2015.1052565

57 (2016) 12743–12754
June

Taylor & Francis
Taylor & Francis Group

Application of immobilized fungi on food efluent treatment using airlift reactor

Rosario Esmeralda Sierra Solache[a], Claudia Muro-Urista[a,*], Rosa Elena Ortega Aguilar[a], Ainhoa Arana Cuenca[b], Alejandro Téllez Jurado[b]

[a]*Department of Chemical Engineering and Research, Technological Institute of Toluca, Avenida Tecnológico s/n Ex-Rancho la Virgen, P.C 52140 Toluca, Mexico, Tel./Fax: +52 01 722 2087224; emails: essme_sh@hotmail.com (R.E. Sierra Solache), cmuro@ittoluca.edu.mx, claudiamuro@hotmail.com (C. Muro-Urista), reortega@ittoluca.edu.mx (R. Elena Ortega Aguilar)*
[b]*Department of Biotechnology, Polytechnic University of Pachuca Carretera Pachuca-Cd, Sahagún Km. 20, Zempoala, Hidalgo, Mexico, Tel. +52 01 7715477510, ext. 2225; emails: ainhoa@upp.edu.mx (A. Arana Cuenca), tejual@upp.edu.mx (A. Téllez Jurado)*

Received 17 July 2014; Accepted 11 May 2015

ABSTRACT

Depuration capacity of immobilized *Phanerochaete chrysosporium* was tested on food effluents which showed high COD, sugar, and nitrogen concentration. Comparative study in airlift reactor and agitated flasks was performed to determine an effective depuration treatment with suspended and immobilized biomass into alginate spheres. While enzymetic production was analyzed in these sytems in order to evaluate influence of the immobilization micro-organism, effluent composition, and aeration mechanism. In addition, the use of seed lettuce allowed evaluating the quality and effectiveness of the studied effluent treatment system. In particular, all tests showed that COD and color of the effluent was reduced by airlift cultures. Treatment achieved a decline in 85% of COD in 120 h. While experiments with 33 PCU color showed high efficiency (100%) in decolourization period of 5 h. Maximum laccase production was mainly found in airlift system with suspended biomass, whereas manganese peroxidase was detected on immobilized micro-organism. Toxicity tests revealed that treated food effluent was not phytotoxic; conversely effluent contains sufficiently high concentrations of nutrients to ensure the germination and lettuce growth.

Keywords: Food effluent; Immobilized biomass; Alginate spheres; Airlift bioreactor; Toxicity

1. Introduction

In recent years, the use of immobilized micro-organisms has been seen as an effective way to enhance the processes of wastewater treatment. Immobilized cells can also promote the production of enzymes and can protect and give stability to micro-organisms [1]. Especially, entrapped fungal cells into matrices may be isolated of toxic organopullants and recalcitrant species compared to free cells [2,3]. The micro-organisms can suspendedly move within their own matrix, consuming substrates that penetrate through of it. Biomass control and shelter within the an area without competition from other microbes present in the wastewater are also seen as advantages of immobilized cells into a container.

In particular form, fungal immobilized prevents cellular activity loss by a higher protection against any

*Corresponding author.

hazardous shocks which could be produced any time by mechanical or chemical means [4–6]. Other advantages as changes in the thixotropic behavior of fluids, separation of fungal biomass from solution, reduction biomass dispersion, and bioaccumulation, as well as, contaminants accumulation from wastewater on walls and clogging in the bioreactors are also exposed in [7,8].

Despite the great benefits provided by the immobilization of cells, there are also various disadvantages of micro-organisms entrapment that have restricted the use of this technology. The main problems of entrapment materials are limiting the mass and oxygen transfer and instability or stress of cells [9]. Besides, mechanical resistance and biodegradation of materials are other drawbacks for the wastewater treatment.

Currently, some of these disadvantages can be overcomed by the use of differents strategies. Composites of organic polymer are studied to improve the material resistance [10], however poor enzyme secretion and toxicity of some material have been revealed; hybrid matrices can inhibit catalytic activity, cell division, and cell growth [1,11]. Mostly, Manganese Peroxidase (*MnP*) production has been limited by encapsulation of *Phanerochaete chrysosporium* in polymeric materials [12–14].

Bioreactors are also considered as an important strategy, which are suitable for the ambient of entrapped micro-organisms [15–17]. Mostly, airlift is recognized as a promising technology for stimulating oxygen transfer into immobilization matrices. Furthermore, low-shear environment and low-energy requirements of airlift are considered with several advantages over other bioreactors. Airlift system can be operated with high loading from effluents and they combine with an efficient mixing in the liquid phase generating by air bubbles using internal or external recirculation loops.

In present study, advantage of composition of a food effluent was tested for the application of a fungal treatment and the production of ligninolytic enzymes for effluent depuration. Viability of biological treatment by immobilized *P. chrysosporium* in Ca-alginate spheres was evaluated in an internal loop airlift bioreactor. Airlift was used to promote good mixing and oxygen transfer particularly into spheres of immobilized biomass. Shake flasks were also utilized to compare the effect of aeration on immobilized micro-organism. Reducing sugars, color reduction, and ligninolytic enzymes produced by *P. chrysosporium* were measured during treatment. In addition, toxicity of treated effluent was also analyzed to evaluate their possible use as irrigation water. Analysis of coupled factors as effluent composition, entrapped micro-organism, and airlift reactor can provide support for the application of encapsulated *P. chrysosporium* for food effluents depuration and reuse.

2. Methodology

2.1. Food effluent samples

Effluents produced by food manufacture process were provided by a food industry localized in Mexico. Intense coloration and turbidity were visible characteristics in the effluents. Presence of colorants mixture and residues of wheat bran, corn bran, reducing sugars and essential amino acids, and vitamins was assumed due to the origin of the effluent.

Important parameters as pH, COD, turbidity, conductivity, sugars, nitrogen concentration, and color were measured to characterize the samples.

2.2. Micro-organism

P. chrysosporium (HEMIM-5) was provided from UAM-CEIB (Biotechnology Research Center) Morelos, México and it was maintained in potato dextrose agar at 28°C. Strain was stored at 4°C and it was cultured every two months.

P. chrysosporium was after incubated on malt extract agar (20% v/v) at 28°C in glass tubes for 5 d. Five milliliters of sterile water were added to tube to scrape and transfer the biomass into 250 mL Erlenmeyer flask containing 50 mL of inoculum medium (AB) composed by food effluent with (%) soluble starch, 0.2; peptone, 0.5; yeast extract, 0.5; $MgSO_4$, 0.02; K_2HPO_4, 0.1.

The flask was incubated at 32°C and 200 rpm in a shaker incubator (Heidolph Unimax 1010) for 24 h for obtaining biomass suspension, which was used as inoculum for immobilization as well as for suspended biomass experiments.

2.3. P. chrysosporium *immobilization*

The immobilization of the fungi was made on alginate spheres. The beads were prepared by adding 2.4 mL of biomass suspension of *P. chrysosporium* to the mixture of 30 mL solution of sodium alginate 3% (p/v) and a sodium solution of carbonate 1%. The mixture was agitated in a Thermo scientific magnetic stirrer HP130915Q and dropped into a $CaCl_2$ 30% solution.

Alginate spheres containing *P. chrysosporium* biomass thoroughly washed with sterile distilled water and preserved in saline (0.9% NaCl solution) at

4℃ for further use. Specific surface areas (SBET), total pore volume V_p, and average pore diameter D_p were also determined by the BET nitrogen adsorption–desorption method (BELSORP-aqua^3, BEL Japan, Inc) on dehydrated spheres.

2.4. Food effluent treatment

Independent tests of food effluents treatment in Erlenmeyer flasks and cylinder internal loop airlift bioreactor (Vichi Airlift Bioreactor FAR-4) were evaluated with suspended biomass and immobilized *P. chrysosporium*.

Flasks of 2 L with a final volume effluent of 1.2 L and airlift bioreactor of 4 L were inoculated in a 10% (v/v) proportion in the suspended biomass experiments, and 0.33% (p/v) proportion in the immobilized fungi.

The flasks were incubated at 170 rpm in an orbital agitator, whereas the reactor was aerated by the draft tube through porous glass diffuser. The experiments were carried out in a batch mode and each experiment was performed with two replicas. An image of the airlift bioreactor used in the present study, is shown in Fig. 1. Fig. 1 describes an airlift consisting in a glass cylindrical vessel of 4 L (7) with two connected sections, a riser and a downcomer, where fluid circulation occurs in a defined cyclic pattern. The vessel contains a concentric draft tube (5) for input and rise of air (8). The downcomer of air is the external cylinder (9).

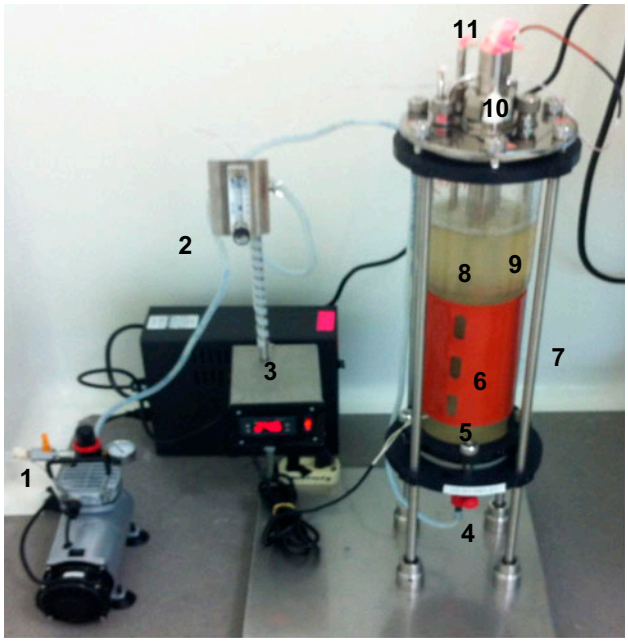

Fig. 1. Airlift bioreactor in batch operation mode. (1) air compressor; (2) rotameter; (3) temperature control; (4) air sparger; (5) draft tube; (6) belt-type heating device; (7) glass reactor; (8 and 9) riser and downcomer; (10) air outlet; (11) oxygen and pH electrodes.

The air is sparged at the bottom, moves upward, and exits at the top of the riser section. The air recirculate through the downcomer section and provide aeration throughout the reactor. The difference in density between riser and downcomer, due to the difference in air holdup, drives the liquid to circulate between the two sections. The liquid velocity was sufficiently high for suspending and recirculating of biomass(suspended or encapsulated) with the liquid. This movement gave as result, a thorough mixing of both biomass and liquid throughout the reactor.

In this case, the input air was controlled at 2 kg/cm^2 through pressure regulating valve localized in air compresser (1). Sterile air was supplied through an air diffuser placed at the bottom of the reactor (4) at an upflow velocity of 1.5–1.8 L/min and the gas flow rate was measured with a rotameter (2).

The reactor was equipped with dissolved oxygen (Hanna DO HI4195) and pH electrodes (Hanna pH HI5333) (11). The temperature in the reactor was controlled using a temperature controller coupled with a belt-type heating device (6) and was monitored in (3).

Airlift and flasks were maintained at 37℃ for the first 48 h of treatment, after this period the temperature was set at 30℃ until the end of the experiment. The temperature change was performed to accelerate the growth speed of *P. chrysosporium* [8].

Samples of treated effluent were collected by a 10 mL sterile pipette in a laminar flow hood every 3 and 24 h during treatment for the suspended and immobilized biomass, respectively. The samples were previously centrifuged for 10 min at 12,000 *g* in a Thermo scientific Espresso 12 centrifuge 11210800 and were taken for the further analysis. Tests on consumption of glucose were performed to confirm adequate porosity and mass transfer in spheres. *P. chrysosporium* biomass, COD, sugars, nitrogen, conductivity, turbidity, and color reduction were also analysed in each case to compare effluent depuration. Enzyme activity was considered to study the effect of the immobilization of *P. chrysosporium* and aeration systems on enzymatic expresión of this micro-organisms. Toxicity treated effluent was analyzed in order to reject toxic secretions of the fungus or toxic intermediates produced by degradation of compounds contained in food effluent.

Concerning spheres of immobilized biomass, spheres were separated from treated effluent and were then washed with deionized water and subsequently

washed with ethanol solution for 15 min for their dehydration. Finally, samples were suspended and dried at −20˚C for 5 min. Later, microscopic examination of spheres was carried out in a SEM (JSM-6610LV, JEOL USA) in a high vacuum mode to analyze their structure and morphology after their use in the experiments.

2.5. Analytical determinations

Wastewater characteristics were determined according to the methods established by Mexican Standards: COD, NMX-AA-030; pH, NMX-AA-008 turbidity, NMX-AA-038; conductivity, NMX-AA-093; total nitrogen, NMX-AA-026 total reducing sugars, NMX-F-496 according to Fehling liquor solution method with a spectrophotometer UV–vis Lambda 36 Perkin Elmer. Real color was monitored according to NMX-AA-045, this wavelength for change of color determination with Hanna colorimeter HI 727 [18].

Fungal biomass was quantified at the end of the experiments as dry weight measurement. The culture was filtered in a fine pore filter paper and dried at 60˚C for 12 h in a stove. Finally, it was placed on silica desiccators and weighed in an Ohaus Pioneer Analytical Balance PA114. Glucose consumption by *P. chrysosporium* was also analyzed by totally reducing sugars.

The ligninolytic enzymes were analyzed as follow. *MnP* activity was determined by monitoring oxidation of phenol red (ε = 4,460/Mcm). The reaction mixture contained 500 μL of supernatant sample, 100 μL 0.1% phenol red, 100 μL of 250 mM sodium lactate (pH 4.5), 200 μL bovine albumin, 50 μL of $MnSO_4$, and 50 μL of 2 mM H_2O_2 in sodium phosphate buffer pH 8.0 [19]. *LiP* activity was determined using veratryl alcohol as substrate. Oxidation of veratryl alcohol was followed by absorbance increase at 310 (ε = 9,300/Mcm). The reaction mixture contained 500 μL of supernatant sample, 1,000 μL of 10 mM citrates buffer pH 3.0, 500 μL of 10 mM veratryl alcohol, and 500 μL of 2 mM H_2O_2 [20]. *Laccase* activity was determined using ABTS as substrate by monitoring absorbance intensification at 420 nm (ε = 36,000/Mcm). The reaction mixture contained 800 μL of supernatant sample and 200 μL of 1.0 mM ABTS in 0.1 M sodium acetate pH 5.0 [21]. The activities were reported as U/L, where one unit of enzyme was defined as the amount of enzyme that oxidized 1 μmol of substrate per minute.

2.6. Toxicity evaluation

The toxicity study of the untreated food effluent and the effluent after the airlift treatment with immobilized fungi was carried out with the purpose of evaluating the toxicity due to the treatment conditions. Toxicity was evaluated on seeds germination and early seedling growth of lettuce seeds (*Lactuca sativa* L). Bioassays were performed by four dilutions of the effluents concentration (100, 75, 50, and 25%) from airlift bioreactor after of 7 d of treatment with immobilized *P. chrysosporium*. Hard water was used to perform the dilutions, and drinking water was used as negative control and positive control as a solution of 1% $ZnSO_4$.

The test consisted of placing 20 lettuce seeds on filter paper disks fine pore in petri dishes with 4 mL effluent sample for a period of 120 h. Seeds were kept in the dark at room temperature (15–20˚C). Shoot height (mm), root lengths (mm), total plant mass (g) and germination index (GI), and a factor of relative seed germination and relative root elongation were determined after this period. In addition, LC_{50} was calculated according Dutkka method [22].

The tests were carried out in triplicate and kept covered with a plastic bag to prevent water evaporation losses. Statistical analysis was conducted using the SAS System Software.

3. Results and discussion

3.1. Food effluent characteristics

The average composition of the effluent industrial gave the following information. Reducing sugars (10.51 g/L), turbidity (52 NTU), conductivity (1,042 μS/cm), total nitrogen (25.6 mg/L) and color (33 PCU), pH (5.85), and COD (4,400 mg O_2/L). The effluent characteristic showed a high COD possibly due to the presence of food preservatives and colorants mixtures. Content of salts in the effluent was confirmed by the high conductivity detected. For the color (dark red), a maximum absorbance at 564 was observed; the color was mainly due to pigments and lignin cellulose residues released during various stages in the food making process. Glucose and nitrogen concentration of effluent was considered also enough as carbon and nitrogen source to ensure the growth of the fungus. It was observable that nitrogen contents in effluent was higher than glucose, approximately in 2/1 ratio.

3.2. P. chrysosporium *immobilization*

Alginate spheres with diameters averaging 5 mm containing *P. chrysosporium* biomass were obtained during fungi immobilization. The diameter was established as an optimal condition for food effluent

treatment due to load and effluent composition. While diameter size was considered due to excessive expanding of *P. chrysosporium* biomass. Data from BET, V_p (0.573 cm^3/g), D_p (5.8701 nm), and SBET (0.468 m^2/g), showed porous spheres and solid structure. Adequate porosity was confirmed by glucose consuming test and excellent growth of micro-organism. Fig. 2, show that about 50% of glucose was consumed by suspended and immobilized fungi during seven days, ending at tenth day.

Alginate beads of the other immobilization systems show differences which include, Dp of 3.81 nm [23] and sphere diameter 3 mm [24]. However, characteristics and differences in size of spheres depend on wastewater composition, volume, and micro-organism.

3.3. Food effluent treatment

Experiments of food effluent treatment showed that nutrients induced efficient production of biomass, effluent depuration, and abundant ligninolytic enzyme secretion. Mean values of two replicate experiments with a standard deviation less than 15% on treated effluent are showed in Table 1. The results correspond to agitated cultures and airlift bioreactor with immobilized and suspended fungal biomass after 7 d of treatment.

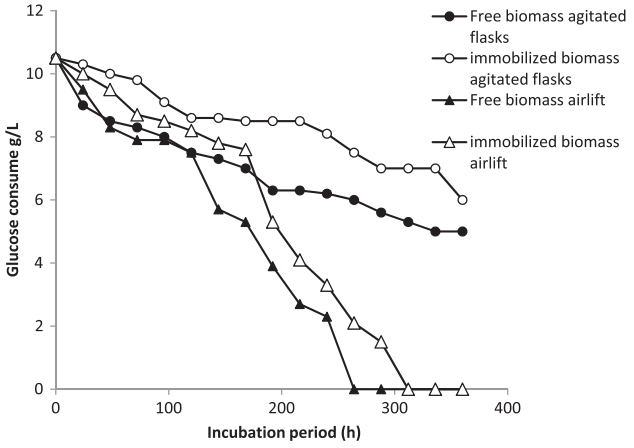

Fig. 2. Consumption of glucose by suspended (free) and immobilized *P. chrysosporium* biomass in airlift and agitated flasks during food effluent treatment.

Abundant growth of *P. chrysosporium* revealed an admirable development of fungi, particularly in airlift reactor. Immobilization limited only 20% biomass production in relation to suspended biomass due to shear stress relatively constant and mild produced in the bioreactor.

Optimum results in effluent depuration were attributed to their composition, therefore, it was assumed that the nutrients in effluent were a substrate potential of *P. chrysosporium*. The glucose and nitrogen concentration was found to be useful for the enhancement of depuration and probably to enzymatic expresión by immobilized micro-organism. In addition, aeration system was effective to reduce the contamination parameters rapidly. Comparative study on the quality of treated effluent in bioreactor confimed this assumption. A great potential of suspended and immobilized *P. chrysosporium* on effluents with 4,400 mg/L COD was detected in airlift experiments. Fungal biomass removed approximately 80–85% over the course of the testes in bioreactor. COD decline (60%) was registered in 120 h of suspended biomass treatment, whereas that encapsulated biomass achieves the same COD removal in 150 h.

Percentage of glucose consumption could be coupled with first growth stage of fungi and COD removal. In this case, decline in glucose and máximum COD removal were deteceted in the same period. Fig. 2, shows a fast decline of glucose by *P. chrysosporium* in airlift in a period of 120–150 h, whereas the amount of glucose consumed was lower in agitated flasks and COD removal was also lower in this agitation system. Nitrogen consumption test was not considered; however, the treated effluent showed 12–13 mg/L as final concentration in all cases,

Table 1
Parameters of quality effluent after 7 d of treatment by immobilized *P. chrysosporium*

Process/biomass	Biomass (g)	COD (mg /L)	pH	Sugars (g/L)	Nitrogen (g/L)	Turbidity (NUT)	Conductivity (μS/cm)	Color (PCU)
Agitated flasks/suspended	6.21	744	5.6	5.6	14.01	3.3	402	3
Agitated flasks/ entrapment	5.02	853	5.4	6.5	15.97	4.5	567	5
Airlift/suspended	7.55	756	5.7	5.9	15.65	4.2	463	0
Airlift/entrapment	5.78	798	5.4	6.1	15.32	4.8	578	0

indicating that it was consumed at the same form by suspended and immobilized fungi.

pH was also considered as a critical parameter governing cell activities during effluent treatment. It is known that the fermentation of carbohydrates to organic acids requires an optimum range of 4.5–6.5; due to pH below 4.5–5 retards or stop the oxidation reactions for the organic acids and thus COD removal may be affected by pH [25].

At the same time, experiments with 33 PCU color showed high efficiency (100%) in decolourization period of 5 h with suspended and immobilized biomass in both treatment types. The results on decolourization could reveal that the colorants were suitable substrate for the peroxidases and oxidases produced by the fungus.

In particular, all tests showed that color of the effluent was reduced by airlift cultures promoting these enzymes due to high oxygen levels. In this case, the removal rate indicated that this effluent treatment was effective for depuration.

The controlled temperatures (30–37˚C) and pH (5–5.7) did not affect COD removal during the treatment. Adding salinity concentration in the effluent did not inhibit fungal growth and effluent depuration into the two aeration systems. In fact, salinity was reduced and probably the salinity and organic content allowed a positive effect due to the association between these components.

Compared COD removal by *P. chrysosporium* on food effluent was higher or similar than other biological treatments. Food effluents are traditionally treated by bioremediation processes including anaerobic and aerobic micro-organisms; the organic load is often eliminated, but other compounds responsible for COD increment are poorly degraded by the organisms normally involved in these treatments, for this reason, to date there are several researches on food wastewater depuration by specific micro-organisms. However *P. chrysosporium* on food effluents treatment has been studied little. Outstanding works on this topic are noted below.

COD removal by suspended and immobilized *Candida tropicalis* (onto a ceramic honeycomb support) in grains-washing wastewater (COD 7,000 mg/L) showed 57 and 76%, respectively [26]. Differences on COD removal were attributed to wastewater, micro-organism-type, and immobilization strategy.

Coupled systems, airlift, and immobilized bacterial consortium was also exposed as suitable treatment of high-carbohydrate wastewater by *C. tropicalis* [25]. Airlift loop reactor tests containing porous ceramic supports achieved a high COD removal (85%), immobilized cells were 4.9 times faster than the suspended cells. Mass transport resistance and concentration gradients apparently did not limit the overall COD removal kinetics. However, in this work, supplying urea as a nitrogen source significantly increased the COD removal from wastewater.

Other microbial treatment on different wastewater containing recalcitrant compounds can be comparable with COD removal by *P. chrysosporium* [8,27–29]. Particularly immobilized bacterial systems with effective depuration are found in [30,31].

Favorable growth of micro-organisms on food residues has also ascertained the influence on some metabolites production. Simultaneously, the micro-organisms have also been successful to treat food wastes with optimal results due to high ratio of degradation [32].

Mostly, information on immobilized *P. chrysosporium* on sugar refinery effluent degradation was found in [33]. The fungal degradation activity was 5–8 and three times greater in terms of decolorization and phenolics reduction with porous carriers than with nonporous carriers. The morphology of the carriers was seen as a factor governing the fungal biodegradation activity.

Besides actuation of immobilized *P. chrysosporium* can also be compared on paper and pulp industrial wastewaters degradation [34]. Significant reduction in COD was attributed to introducing sucrose and ammonium chloride.

Degradation of specific contaminants by immobilized cell culture has also been associated to bioreactor. Some strains such as URM 6181 of *P. chrysosporium* immobilized in polyurethane foam (PUF) [35], *Bacillus-mycoides* on polyvinyl alcohol (PVA)-sodium alginate-Kaolín [36], and *Bacillus fusiformis* (BFN) strain on alginate–PVA–clays [37] showed advantageous degradation in airlift bioreactor.

In each case, the excellent results about depuration action from micro-organisms are associated to microbe characteristics and culture conditions; however, many researchers agree on the importance of nutrients that should be present during the culture processes.

3.4. *Enzyme activity of* P. chrysosporium

Fig. 3 shows the maximum enzyme activity (U/L) of *MnP*, *LiP*, and *Laccase* for each process. Maximum activities are reported in a period of 240 h, when COD does not have significant changes.

Different enzymatic profiles by suspended and entrapped biomass were observed in different agitation processes with high enzymatic activities. However, maximum peak of enzymes was more evidenced

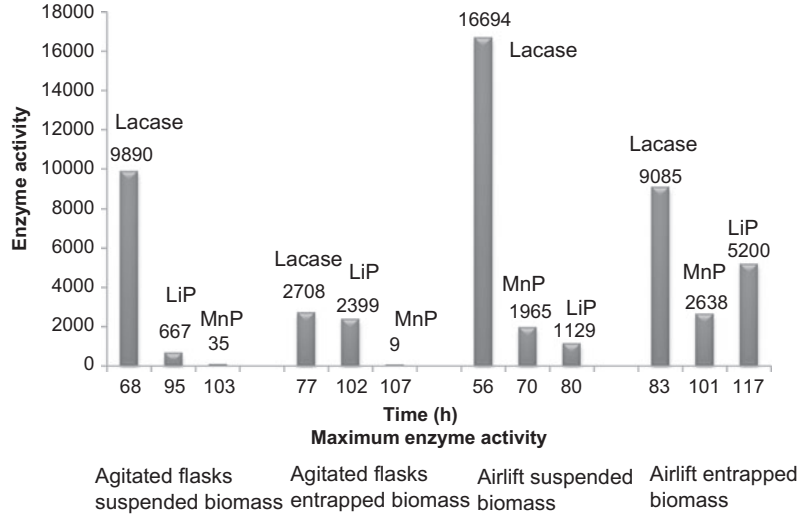

Fig. 3. Maximum enzyme production by suspended and entrapped *P. chrysosporium* biomass during food effluent treatment in agitated flasks and airlift bioreactor.

in airlift. Comparative results from immobilized and non-immobilized *P. chrysosporium* showed similar enzymatic actuation.

Amazing enzyme *Laccase* secretion was detected in suspended biomass. Tests showed maximum peak *Laccase* (9,890 U/L) at 68 h in agitated flasks and (16,694 U/L) in airlift at 56 h after starting the fungus cultivation, whereas immobilized biomass produced less *Laccase* in a time largest than suspended biomass. It may indicate that the immobilization affected the diffusivity of this enzyme or *Laccase* production was limited by biomass immobilization.

In contrast, data to *MnP* and *LiP* showed that maximum activities of these enzymes were produced by immobilized biomass. *LiP* (5,200 U/L) was detected in this system after of 117 h of effluent treatment, whereas maximum peak *MnP* secretion (2,638 U/L) was found at 101 h. These results indicated that production of *LiP* and *MnP* is generally optimal at high oxygen tension, but these enzymes can be inhibited in suspended biomass by agitation in airlift. Increment of *MnP* enzyme production by immobilized biomass can be associated to contact area between cells and oxygen without sheer stress compared with suspended mycelia. Higher activity from immobilized micro-organism may also be attributed to shorter cell generation time, cell retraction, and differentiation, due to encapsulation which is characterized by the increases in the enzymatic and metabolite transport. Additionally, the role of calcium in alginate immobilization could be considered as induced elicitation and thus, increments the productivity of cells is a response of this stimule. This phenomena was studied in plant cells [38], however actually this topic is not still documented on cell micro-organisms.

Ligninolytic enzymatic ability of *P. chrysosporium* has been often tested on detoxification of textil effluents and on individual models of wastewater containing synthetic dyes as well as toxic and recalcitrants contaminants. Degradation efficiences have been associated to different conditions under which it is incubated. To regard, there are many differences and controversuial results on this topic. Nutrient limiting conditions, addition of inducers that enable enzyme production, and immobilization techniques between other have been studied to give response to many questions on enzyme production of this micro-organism [39]. Stupendous results show that immobilizated *P. chrysosporium* on PUF can achieve a maximum *MnP* activity of 915.62 U/L on eighth day [40]. While 421 U/L after 200 h of fermentation was detected in cultures containing carbon and nitrogen sources and addition of tween 80 (0.05%, v/v) and Mn^{2+} (174 M) [41,42]. In these tests, limitation of nitrogen was associated with maximum *MnP* activity.

Immobilization on alginate beads has also been exposed in turn to enhance *MnP* production on decolourization of different azo dye [1,43]. Authors used synthetic cultures supplemented with glucose and ammonium to stimulate enzymes secretion on dyes. However, tests revealed a constant of decolourization kinetic (K_{day}) and enzyme secretion low compared to suspended biomass. The result was attributed mainly to colorants-type and entrapment material which might act as a barrier for immediate dissociation of the azo dyes.

Treatment of industrial wastewater including those producing sugar refinery, olive oil and pulp, and paper mill effluent can be found in [33,34,44]. These reports coincided in that immobilized *P. chrysosporium* was more efficient than suspended cells since ligninolytic enzymes of *P. chrysosporium* are very sensitive to shear stress. Immobilization in a porous supports or entrapment beads protected the enzymatic system against the detrimental effect of shear forces thereby improving the fungus activity. This is the most probable explanation for the high activity observed in cultures containing biomass immobilized.

Added relevant report on high enzyme production by other white rot fungi can be reviewed in [45]. Laccase (1,000 U/L) by *Trametes hirsuta* immobilized into alginate was achieved by addition of fresh ammonium chloride on prepared culture medium. Tests were performed using 40 dyes and maximum laccase was detected in 20 d of fermentation. Current results are also found in [46]. This report shows a high enzyme secretion by strains of basidiomycetes on various food residues (lignocellulosic substrates). Maximum activity was revealed on day five and nine; all species of the genus *Trametes* expressed comparatively high *Laccase* activity (192–61,488 U/L), while *Phellinus robustus* was a promising producer of *MnP*, accumulating more than 4,000 U/L of enzyme activity. This behavior was attributed to food residues with different nitrogen sources.

Contribution by substrates from food residues in the production of other fungal metabolites are also exposed in current researches [47,48]. Degradation of food residues for obtaining biomolecules via fermentation was dependent on nitrogen, and was considered as a source essential in these processes.

In order to achieve an effective continuous effluent treatment with *P. chrysosporium*, in the present report, it was demonstrated that effluent composition contributed on an efficient production of ligninolytic enzymes and therefore, effluent depuration was achieved by biological treatment. In addition, the immobilization of fungus in alginate spheres and use of airlift reactor were a promising form to air supply and to stimulate cell metabolism with an effective effluent treatment.

3.5. Operational stability of spheres after effluent treatment

Spheres of immobilized biomass of *P. chrysosporium* provided a stupendous microenvironment for the cells to proliferate. Spheres also exhibited solid and porous structure and were resistant to speed in agitated cultures and airlift. The material showed a greater strength and saturated wet density even after its use. Clogging problems, which would hinder mass and oxygen transfer rate were not presented by leakage biomass. Added, the surface of spheres did not show biomass leakage even after its use.

Air bubbles allowed the movement of spheres throughout the airlift during effluent treatment due to lightness of material spheres. The level of shear stress by airlift did not effect cell viability into matrix, aggregation was not observed in micrographs.

These results indicated a very high operational stability of the immobilized biomass of *P. chrysosporium* in alginate material; however, the spheres were only used in a cycle of effluent treatment and other use cycles or their reuse was not considered.

Fig. 4 shows micrographs of dry alginate spheres containing *P. chrysosporium* biomass before effluent treatment and after seven days of use in airlift. Micrographs revealed spheres of compact structure and high porosity (transversal cut) after their dehydration and use.

Alginate is recognized as suitable material for the immobilization cells in many reviews on this topic [49]; however, significant fragility (due to sensitivity to chelating agents and non-gelling ions) affects the efficacy of matrices as entrapment material. Thus, Ca-alginate is not a suitable method for most field scale applications in wastewater treatment [11]. Despite this disadvantage, properties as biodegradability, hydrophilicity, presence of carboxylic groups, low density, and stability over an experimental pH, have motivated interest to continue with the development of systems with entrapped cells into hybrid matrices of alginate. The combination of alginate with other polymers is now studied to improve the material properties as mechanical stability and as well as suitable diffusion to wear nutrients into the cells and diffusion of the degradation products released by the micro-organisms. However, stability/viability of cells or biomolecules such as enzymes have been attributed to influence of alginate in hybrid entrapped materials [8,50]. Therefore, this research can be seen as a base to achieve successful processes of immobilized cell technology in the field of wastewater treatment. Alginate continue in study and thus, one might expect increased production of enzymes in hybrid entrapment material and airlift as aeration system.

3.6. Toxicity of treated effluent

The use of toxicity test on seed lettuce allowed evaluating the quality and effectiveness of the studied food wastewater treatment system in airlift reactor with immobilized *P. chrysosporium*.

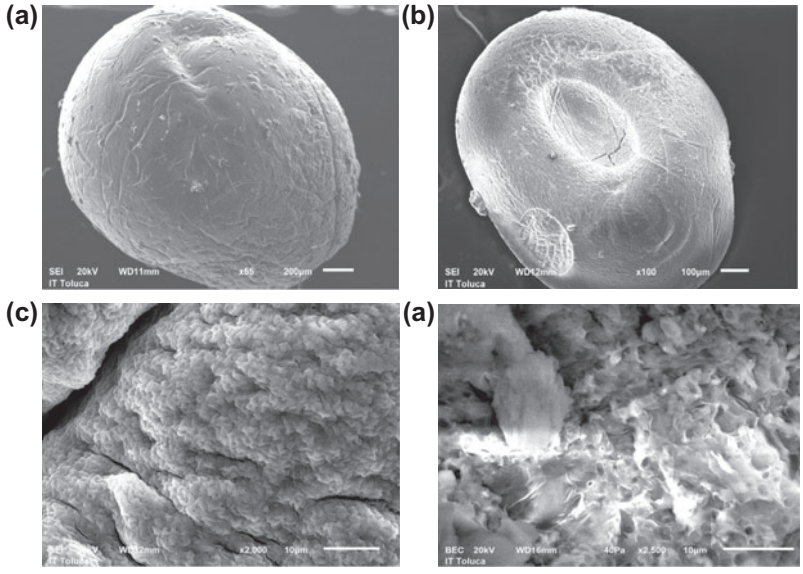

Fig. 4. Micrograph of encapsulate *P. chrysosporium* in alginate spheres. (a) Dehydrated sphere after of encapsulation of *P. chrysosporium*; (b) Dehydrated sphere with *P. chrysosporium* after the seventh day of effluent treatment in airlift; (c) Transversal cut of a dehydrated sphere of alginate; (d) Transversal cut of a dehydrated sphere of alginate with *P. chrysosporium* biomass.

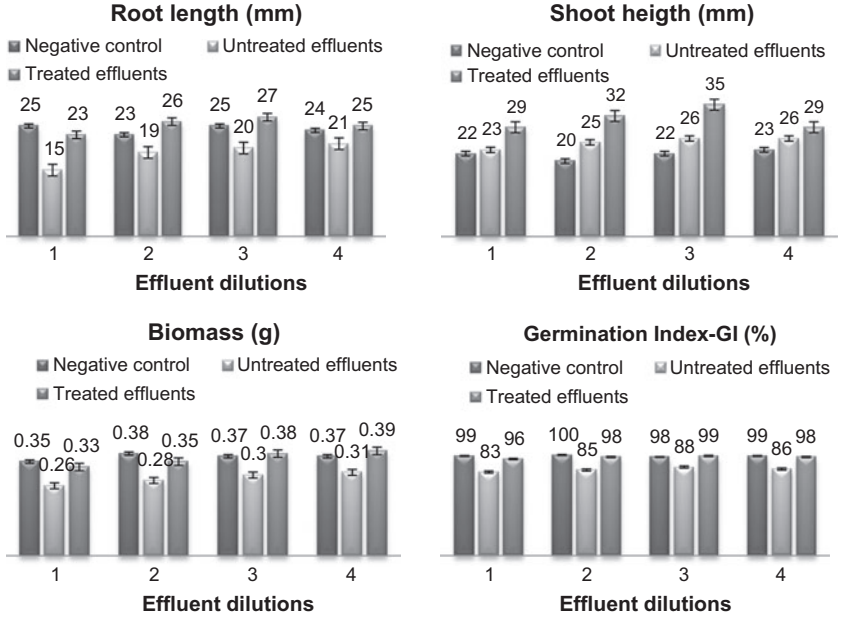

Fig. 5. Relative growth of root length, shoot height, biomass, and GI for different effluent dilutions (100, 75, 50, and 25%) of untreated effluent of treated effluent and negative control.

Toxicity tests revealed that treated food wastewater was not phytotoxic; conversely effluent contains sufficiently high concentrations of nutrients to ensure the germination and lettuce growth.

Fig. 5 shows data obtained from toxicity tests in lettuce exposed to different concentrations of effluent before and after the fungi treatment in airlift and encapsulated *P. chrysosporium* in alginate spheres.

All samples of treated effluent did not inhibit the seed germination, since it achieved similar results in comparison with the negative control (90%). High seed germination of lettuce could be understood by null toxicity of effluent; however, this effect has also been attributed to low sensitivity of lettuce to toxicants [51,52], for this reason other parameters should be determined to confirm the effluents toxicity on this plant.

In this case, no toxicity of treated effluent was also confirmed by the growth of lettuce roots and the biomass results, since these two parameters were also not inhibited by the composition of the effluent.

Particularly stimulation in the growth of roots and shoots of lettuce with the effluent concentration of 100–50% was observed in all tests. However, shoot height exposed to a concentration of 50% of treated effluent showed the highest growth (18% longer than in the control sample).

Regarding biomass of lettuce, it was found that the total biomass is increased by exposure to effluent treated food. Concentration of 100 and 50% of the treated effluent, reached 27 and 30% greater biomass, respectively, compared with the control sample. In addition, the average lethal concentration (LC_{50}) was insignificant, confirming that treated effluents were nontoxic. These results can be attributed to nontoxic metabolites production of immobilized *P. chrysosporium* during wastewater treatment.

Other current research demonstrated an increase in toxicity (and/or ecotoxicity) of treated textile effluent by *P. chrysosporium* URM 6181 strain. The fungus showed degradation products seem to be more toxicants than the original compounds [35]. Effluent treated accumulated a mutagenic metabolite derived from indigo dye. However, this result is understandable due to presence of toxic dyes of textil industries.

4. Conclusions

Comparative study on industrial food effluent treatment by two different aereation systems, airlift, and shake flasks, both with suspended and immobilized biomass of *P. chrysosporium* showed that conjugate factors as effluent composition, aeration systems in airlift, and encapsulation of fungi, promoted the development of the micro-organism and enzyme production with high depuration percentage on effluent.

Consume glucose test revealed an adequate mass transport on fungi immobilized biomass, due to aeration system in airlift and operation conditions. Relative results on effluent depuration in airlift indicated that immobilized biomass reduced 80–85% of COD and total color of food effluent.

High level of enzyme activities was also found in airlift, particularly *MnP* was detected in immobilized system, whereas that elevated *Laccase* activity and *LiP* was found in suspended biomass. The effluent depuration was not associated with a single enzyme.

In addition toxicity tests revealed that treated effluent was not phytotoxic on lettuce seeds; conversely effluent contains sufficiently high concentrations of nutrients to ensure the germination and lettuce growth.

The feasibility of application of entrapped biomass of *P. crysosporium* in alginato spheres for depuration of food effluent in an airlift bioreactor was tested; however, more studies on other alginate composites are necessary to achieve industrial application. Coupled systems of immobilized cell technology and airlift reactor could have a great potential in the field of wastewater treatment.

References

[1] K.V. Radha, I. Regupathi, A. Arunagiri, T. Murugesan, Decolorization studies of synthetic dyes using *Phanerochaete chrysosporium* and their kinetics, Process Biochem. 40 (2005) 3337–3345.
[2] P.P. Champagne, J.A. Ramsay, Reactive blue 19 decolouration by laccase immobilized on silica beads, Appl. Microbiol. Biotechnol. 77 (2007) 819–823.
[3] D. Gao, L. Du, J. Yang, W. Wu, H. Liang, A critical review of the application of white rot fungus to environmental pollution control, Crit. Rev. Biotechnol. 30 (2010) 70–77.
[4] I. Mielgo, M.T. Moreira, G. Feijoo, J.M. Lema, A packed-bed fungal bioreactor for the continuous decolourisation of azo-dyes (Orange II), J. Biotechnol. 89 (2001) 99–106.
[5] O. Yesilada, D. Asma, S. Cing, Decolorization of textile dyes by fungal pellets, Process Biochem. 38 (2003) 933–938.
[6] P. Kaushik, A. Malik, Fungal dye decolourization: Recent advances and future potential, Environ. Int. 35 (2009) 127–141.
[7] B. Xin, Y. Xia, Y. Zhang, H. Aslam, C.H. Liu, S. Chen, A feasible method for growing fungal pellets in a column reactor inoculated with mycelium fragments and their application for dye bioaccumulation from aqueous solution, Bioresour. Technol. 105 (2012) 100–105.
[8] D.F. Gonzalez-Ramírez, C. Muro-Urista, A. Arana-Cuenca, A. Téllez-Jurado, A.E. González-Becerra, Enzyme production by immobilized *Phanerochaete chrysosporium* using airlift reactor, Biologia 69 (2014) 1464–1471.
[9] Y. Cohen, Biofiltration-the treatment of fluids by microorganisms immobilized into the filter bedding material: A review, Bioresour. Technol. 77 (2001) 257–274.
[10] K. Zhang, Y. Xu, X. Hua, H. Han, J. Wang, J. Wang, Y. Liu, Z. Liu, An intensified degradation of phenanthrene with macroporous alginate–lignin beads immobilized *Phanerochaete chrysosporium*, Biochem. Eng. J. 41 (2008) 251–257.

[11] L.E. de-Bashan, Y. Bashan, Immobilized microalgae for removing pollutants: Review of practical aspects, Bioresour. Technol. 101(2010) 1611-1627.
[12] K. Enayatzamir, H.A. Alikhani, B. Yakhchali, F. Tabandeh, S. Rodríguez-Couto, Decolouration of azo dyes by *Phanerochaete chrysosporium* immobilised into alginate beads, Environ. Sci. Pollut. Res. 17 (2010) 145–153.
[13] K. Pakshirajan, A. Sivasankar, N.K. Sahoo, Decolourization of synthetic wastewater containing azo dyes by immobilized *Phanerochaete chrysosporium* in a continuously operated RBC reactor, Appl. Microbiol. Biotechnol. 89 (2011) 1223–1232.
[14] J. Urra, L. Sepúlveda, E. Contreras, C. Palma, Screening of static culture and comparison of batch and continuous culture for the textile dye biological decolorization by *Phanerochaete chrysosporium*, Braz. J. Chem. Eng. 23 (2006) 281–290.
[15] P. Blánquez, M. Sarrà, T. Vicent, Development of a continuous process to adapt the textile wastewater treatment by fungi to industrial conditions, Process Biochem. 43 (2008) 1–7.
[16] A. Anastasi, F. Spina, V. Prigione, V. Tigini, P. Giansanti, G.C. Varese, Scale up of a bioprocess for textile wastewater treatment using *Bjerkandera adusta*, Bioresour. Technol. 101 (2010) 3067–3075.
[17] A. Anastasi, F. Spina, A. Romagnolo, V. Tigini, V. Prigione, G.C. Varese, Integrated fungal biomass and activated sludge treatment for textile wastewaters bioremediation, Bioresour. Technol. 123 (2012) 106–111.
[18] Secretaria de Economía, Dirección General de Normas, Catálogo de Normas Mexicanas (NMX), (2014). Available from: www.economia.gob.mx.
[19] R.C. Minussi, S.G. de Moraes, G.M. Pastore, N. Duran, Biodecolorization screening of synthetic dyes by four white-rot fungi in a solid medium: Possible role of siderophores, Lett. Appl. Microbiol. 33 (2001) 21–25.
[20] K. Sen, K. Pakshirajan, S.B. Santra, Modeling the biomass growth and enzyme secretion by the white rot fungus *Phanerochaete chrysosporium*: A stochastic-based approach, Appl. Biochem. Biotechnol. 167 (2012) 705–713.
[21] D. Pant, A. Adholeya, Identification, ligninolytic enzyme activity and decolorization potential of two fungi isolated from a distillery effluent contaminated site, Water Air Soil Pollut. 183 (2007) 165–176.
[22] B. Dutkka, Short-Term Root Elongation Toxicity Biossay. Methods for Toxicological Analysis of Waters, Wastewaters and Sediments, National Water Research Institute Environment, Canada, Burlington, Ontario, 1989
[23] Y. Lu, Z. Jiang, S. Xu, H. Wu, Efficient conversion of CO_2 to formic acid by formate dehydrogenase immobilized in a novel alginate–silica hybrid gel, Catal. Today 115 (2006) 263–268.
[24] P.C. Peart, A.R.M. Chen, W.F. Reynolds, P.B. Reese, Entrapment of mycelial fragments in calcium alginate: A general technique for the use of immobilized filamentous fungi in biocatalysis, Steroids 77 (2012) 85–90.
[25] K. Zhang, Y. Xu, X. Hua, H. Han, J. Wang, J. Wang, Y. Liu, Z. Liu, An intensified degradation of phenanthrene with macroporous alginate-lignin beads immobilized *Phanerochaete chrysosporium*, Biochem. Eng. J. 41 (2008) 251–257.
[26] Y. Zhang, B.E. Rittmann, J. Wang, Y. Sheng, J. Yu, H. Shi, Y. Qian, High carbohydrate wastewater treatment by IAL-CHS with immobilized *Candida tropicalis*, Process Biochem. 40 (2005) 857–863.
[27] M.N. Sepehr, S. Nasseri, M. Zarrabi, M.R. Samarghandi, A. Amrane, Removal of Cr(III) from tanning effluent by *Aspergillus niger* in airlift bioreactor, Sep. Purif. Technol. 96 (2012) 256–262.
[28] Z. Jemaat, M.E. Suárez-Ojeda, J. Pérez, J. Carrera, Partial nitritation and *o*-cresol removal with aerobic granular biomass in a continuous airlift reactor, Water Res. 48 (2014) 354–362.
[29] Z. Chen, Z. He, C. Tang, D. Hu, Y. Cui, A. Wang, Y. Zhang, L. Yan, N. Ren, Performance and model of a novel multi-sparger multi-stage airlift loop membrane bioreactor to treat high-strength 7-ACA pharmaceutical wastewater: Effect of hydraulic retention time, temperature and pH, Bioresour. Technol. 167 (2014) 241–250.
[30] A. Kunamneni, T. Prabhakar, B. Jyothi, P. Ellaiah, Investigation of continuous cyclodextrin glucanotransferase production by the alginate-immobilized cells of alkalophilic *Bacillus sp*. in an airlift reactor, Enzyme Microb. Technol. 40 (2007) 1538-1542.
[31] S. He, Y. Lin, K. Hou, S.J. Hwang, Degradation of dimethyl-sulfoxide-containing wastewater using airlift bioreactor by polyvinyl-alcohol-immobilized cell beads, Bioresour. Technol. 102 (2011) 5609–5616.
[32] F. Vendruscolo, J.L. Ninow, Apple pomace as a substrate for fungal chitosan production in an airlift bioreactor, Biocatal. Agric. Biotechnol. 3 (2014) 338–342.
[33] C. Guimarães, C. Matos, J. Azeredo, M. Mota, R. Oliveira, The importance of the morphology and hydrophobicity of different carriers on the immobilization and sugar refinery effluent degradation activity of *Phanerochaete chrysosporium*, Biotechnol. Lett. 24 (2002) 795–800.
[34] V. Gomathi, A. Ramanathan, N. Sivaramaiah, V.R. Ramanjaneya, D. Jayasimha, Decolourization of paper mill effluent by immobilized cells of *Phanerochaete chrysosporium*, Int. J. Plant Anim. Environ. Sci. 2 (2012) 141–146.
[35] R.C.M. Miranda, E. Gomes, N. Pereira, M.A. Marin-Morales, K.M. Machado, N. Gusmão, Biotreatment of textile effluent in static bioreactor by *Curvularia lunata* URM 6179 and *Phanerochaete chrysosporium* URM 6181, Bioresour. Technol. 142 (2013) 361–367.
[36] H. Lin, Z. Chen, M. Megharaj, R. Naidu, Biodegradation of TNT using *Bacillus mycoides* immobilized in PVA-sodium alginate–kaolin, Appl. Clay Sci. 83-84 (2013) 336–342.
[37] Ch Lin, L. Gan, Z. Chen, M. Megharaj, R. Naidu, Biodegradation of naphthalene using a functional biomaterial based on immobilized *Bacillus fusiformis* (BFN), Biochem. Eng. J. 90 (2014) 1–7.
[38] W.R. Curtis, P. Wang, A. Humphrey, Role of calcium and differentiation in enhanced sesquiterpene elicitation from calcium-alginate immobilized plant tissue, Enzyme Microb. Technol. 17 (1995) 554–557.
[39] D. Gao, L. Du, J. Yang, W. Wu, H. Liang, A critical review of the application of white rot fungus to environmental pollution control, Crit. Rev. Biotechnol. 30 (2010) 70–77.

[40] H. Liang, Y.G. Zeng, D.W. Gao, Enzymes production by *Phanerochaete chrysosporium* immobilized on different carriers, J. Biotechnol. 136 (2008) S508.
[41] R.O. Ürek, N.K. Pazarlioğlu, A novel carrier for *Phanerochate chrysosporium* immobilization, Artif. Cells Blood Substit. Immobil. Biotechnol. 32 (2004) 563–574.
[42] S. Sayadi, F. Zorgani, R. Ellouz, Decolorization of olive mill waste-waters by free and immobilized *Phanerochaete chrysosporium* cultures, Appl. Biochem. Biotechnol. 56 (1996) 265–276.
[43] M. Zahmatkesh, F. Tabandeh, S. Ebrahimi, Biodegradation of reactive orange 16 by *Phanerochaete chrysosporium* fungus: Application in a fluidized bed bioreactor, Iran J. Environ. Health 7 (2010) 385–390.
[44] V. Gomathi, A. Ramanathan, N. Sivaramaiah, V.R. Ramanjaneya, D. Jayasimha, Decolourization of paper mill effluent by immobilized cells of *Phanerochaete chrysosporium*, Int. J. Plant Anim. Environ. Sci. 2 (2012) 141–146.
[45] A. Domínguez, S. R. Couto, M.A. Sanromán, Dye decolorization by *Trametes hirsuta* immobilized into alginate beads, World J. Microbiol. Biotechnol. 21 (2005) 405–409.
[46] G. Songulashvili, V. Elisashvili, S. Wasser, E. Nevo, Y. Hadar, Basidiomycetes laccase and manganese peroxidase activity in submerged fermentation of food industry wastes, Enzyme Microb. Technol. 41 (2007) 57–61.
[47] L. Jiang, S. Pan, J.M. Kim, Influence of nitrogen source on chitosan production carried out by *Absidia coerulea* CTCC AF 93105, Carbohydr. Polym. 86 (2011) 359–361.
[48] A. Cardoso, C.I.M. Lins, E.R. dos Santos, M.C.F. Silva, G.C. Campos-Takaki, Microbial enhance of chitosan production by *Rhizopus arrhizus* using agroindustrial substrates, Molecules 17 (2012) 4904–4914.
[49] D. Rodrigues, T.A.P. Rocha-Santos, A.C. Freitas, A.M.P. Gomes, A.C. Duarte, Strategies based on silica monoliths for removing pollutants from wastewater effluents: A review, Sci. Total Environ. 461-462 (2013) 126–138.
[50] M. Perullini, M. Jobbágy, N. Mouso, F. Forchiassin, S.A. Bilmes, Silica–alginate-fungi biocomposites for remediation of polluted water, J. Mater. Chem. 20 (2010) 6479–6483.
[51] J. Zaltauskaitė, R. Vaisiūnaitė, Evaluation of municipal effluent toxicity using higher plants and invertebrates, Environ. Res. Eng. Manage. 3 (2010) 17–23.
[52] J. Charles, B. Sancey, N. Morin-Crini, P.M. Badot, F. Degiorgi, G. Trunfio, G. Crini, Evaluation of the phytotoxicity of polycontaminated industrial effluents using the lettuce plant (*Lactuca sativa*) as a bioindicator, Ecotoxicol. Environ. Saf. 74 (2011) 2057–2064.