

Pb(II) removal from aqueous systems by biosorption-flotation on mycelial residues of *Penicillium chrysogenum*

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Abstract: The aqueous systems arising from mining industry or mineral and metal processing, have a complex composition so they require the application of some efficient and economic purification methods. Lead is known as one of the most toxically metals for the environment protection and in the mean time, one with a high reserve depletion rate.

A relative recent interest among the separation methods presents the biosorption coupled with flotation, a high performant method named biosorption-flotation. Using the mycelial residues treated on different ways as sorbent materials the method assure an ecologically and economically efficiency.

The paper presents a study regarding Pb(II) removal from aqueous systems on a wide range of concentration (5-1000 mg·L⁻¹) by biosorption-flotation with mycelial wastes of *Penicillium*. The main parameters of the biosorption process were studied: pH solution, Pb(II) concentration, biomass concentration. Dissolved air flotation was applied for the loaded inactive biomass separation from the aqueous solution and the following parameters were studied for the biosorption-flotation process: pH, sorbent- Pb (II) contact time and Pb (II) concentration in order to obtain a high separation (over 95%) of the loaded biomass.

The biosorption-flotation process offer the possibility of high Pb (II) removals (R% = 85-99).

1 INTRODUCTION

Although lead pollution from mining activities represents a relatively localized problem, its magnitude is significant, and particularly on the water pollution side.

The toxicology of lead has been extensively studied. Inorganic lead (Pb²⁺) is a general metabolic poison. One of the most insidious effects of inorganic lead is its ability to replace calcium in bones and remain there to form a reservoir for long-term release.

It is estimated that about 180.000 tons/year of lead is mobilized by the natural weathering process and approximately 2 millions tons is mined yearly. Around 10% of this total is lost in treatment of the ore to produce concentrate, meaning that the amount of lead discharged in the environment is equal to the amount weathered from igneous rocks (Volesky, 1990).

Treatment of wastewaters containing lead means, for the very most plants, precipitation followed by sedimentation. The costs involved by this kind of treatments are often higher than the penalties. Thus, with continued extraction of

mineral resources and environmental accumulation of lead, greater efficiency and recovery must be devised. In this respect the processes using inactive biomass are for an increasing interest, the wasted biomass being of wide variety and cheap.

It was shown that these biological materials can accumulate heavy metals in their walls, even that de binding sites for chelating were not identified. The effect of lead on microorganisms has been extensively studied, mainly with living cultures of bacteria. Relatively few studies focused on fungi. Of the filamentous fungi, *Rhizopus arrhizus* was shown to sequester a relatively high quantity of lead (0,5 mmol Pb(II)/g; Tobin et al, 1984) and some other heavy metal ions (Fourest & Roux, 1992).

If the biosorption process is operated in stirred tanks using a suspended biomass (Jackson, 1992), a subsequently solid/liquid separation stage is required. The specific characteristics of this kind of sorbat/sorbent system make difficult the separation by filtration (the process needs more time and may face filter blocking problems especially in the case of fine or ultrafine particles), centrifuging (apparent more expensive) or sedimentation (relatively slow process inadequate to biological materials which are usually of low density). Some flotation techniques were applied for microorganisms separation (Gadd, 1988) and the possibility of combining biosorption and flotation were also studied (Zouboulis & Matis, 1996). Thus, flotation became of great interest among the bioseparation processes.

The aim of the present paper is to study the ability of *Penicillium chrysogenum* by-product of pharmaceutical industries for lead removal from aqueous solutions and also to separate by flotation (DAF technique) the loaded biomass.

2 MATERIALS AND METHODS

2.1 Reagents

The fungal biosorbent used for this study was a by-product (mycelial residues of *P. chrysogenum*) of pharmaceutical industries. The mycelial wastes were previously treated by repeated washing and subsequently dried in two different ways: underpressure drying and ketone drying; the size particles was less than 100 μ .

Model solutions of different concentrations were prepared by dissolving Pb(NO₃)₂, purchased from Merck.

For pH adjusting were used solutions of NaOH or HCl p.a. (0,1 M).

2.2 Methods

The biosorption experiments were carried out in batch system. The samples of Pb(II) solutions were shaken together with corresponding quantities of biomass

in 50 ml flasks on a Braun shaker at a low constant rate (150 rpm) , until the equilibrium was reached (24 h).

The flotation process was carried out in a dissolved-air-flotation apparatus (Stoica, 1997). The stirring of model mixtures (300 ml), containing the lead solution and the biomass was carried on by an electrical stirrer. Water saturated with air in the saturator and kept under pressure of 4.10^5 N/m^2 was introduced to the base of the cell. When releasing water to the atmospheric pressure, fine air bubbles were generating, appropriated for solid/liquid separation.

Sorption capacities increase along with initial Pb(II) concentrations, as long as binding sites are not saturated. In the range of low concentrations (under 100 mg/l), the biomass presents similar sorption capacities but in the range of high initials concentrations (over 500 mg/l) we observed a lowering of Pb(II) retention on the Penicillium biomass treated by underpressure drying.

2.3 Equipments

Chemical analyses by AAS of the remaining solutions were used in order to assay the unremoved cadmium or lead.

For pH regulation we used an ORION A 290 pH-meter.

IR spectra of biomass before and after the biosorption-flotation process was registered by an IR-20 spectrophotometer. The high particulated samples were mixed with KBr and then pressed into a transparent disk. A pure KBr disk was used as a blank. Using this method, samples do not disperse radiation for 400-5000 cm^{-1} wavenumber range.

3 RESULTS AND DICUSSIONS

3.1 Sorption equilibrium

Biosorption of Pb(II) species from aqueous solutions on inactive biomass occurs by sorption mechanisms. In Figure 1, equilibrium isotherms for Pb(II) biosorption (without pH controlling) on inactive Penicillium biomass treated in two different ways, are presented. It is obviously that Langmuir type isotherms describe well the biosorption process. The respective equation can be written as:

$$q_e = \frac{bq_m C_{eq}}{(1 + bC_{eq})} \quad (1)$$

where C_{eq} represents the free metallic ions concentration at equilibrium, and b , q_m are empirical constants, characterizing the metal-biomass interactions.

3.2 Sorption parameters

The presence of ionic lead species in aqueous solutions strongly depends on solution pH and also on the initial concentrations of the solutions. Thus, the pH is an important parameters for the biosorption process (Figure 2 and 3) leading to different sorption capacities and removal efficiencies of lead. Experimental data plotted in Figure 2 and 3 show similar alure of the curves for the four studied Pb(II) solutions of different concentrations. The maximal sorptions occur for the interval of pH = 4-5.

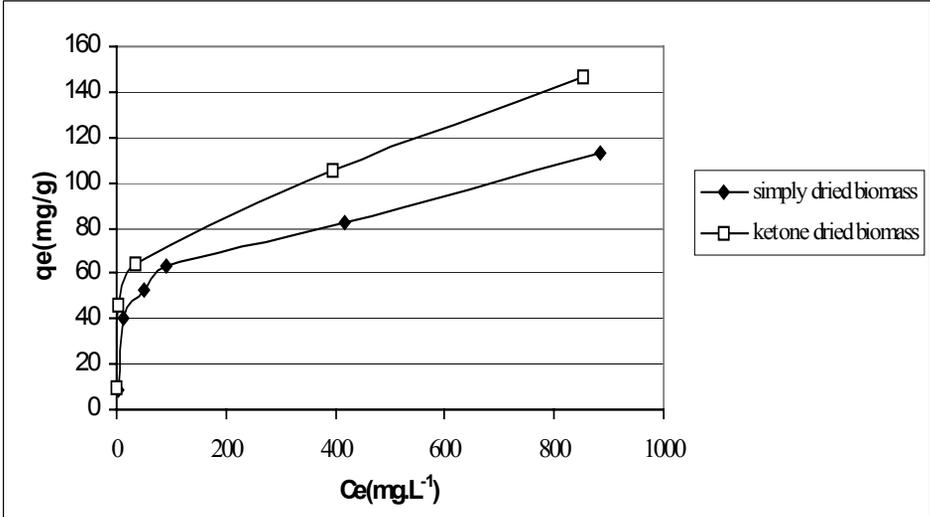


Figure 1 Sorption equilibrium isotherm
biomass amount=1g/l; sorption time=24h

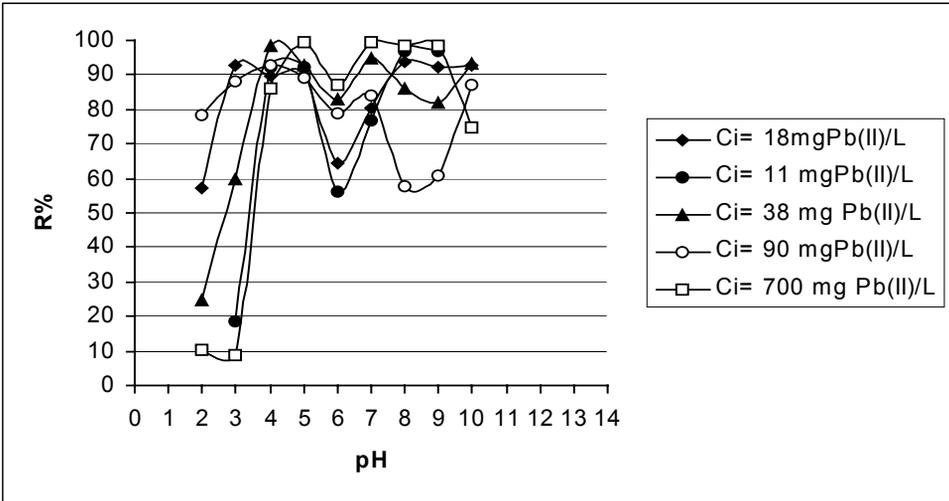


Figure 2 Influence of solution pH on biosorption process
sorption time = 24h, biomass amount = 2g/l; underpressure dried biomass

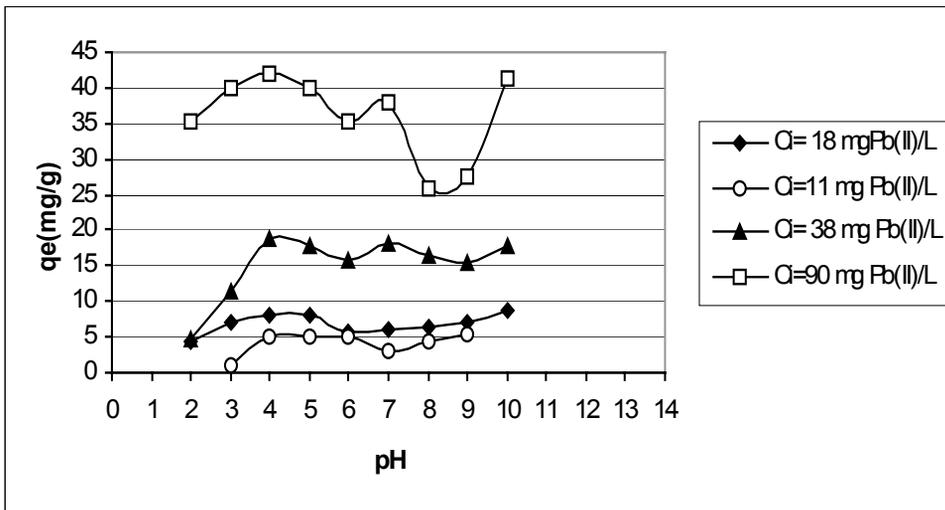


Figure 3 Pb(II) recovery (R%) as a function of solution pH
sorption time =24h, biomass amount=2g/L; underpressure dried biomass

Expressing the same experimental data in terms of removal efficiency permitted us the observation: there are two domains of pH where the lead removal is maximum (pH= 4-5 and pH=7-10). In fact, the sorption occurs only up to pH=5,5-6 (depending on Pb(II) initial concentration), after this value beginning an important precipitation of lead as $Pb(OH)_2$. It is obviously a decreasing of Pb(II) removal around pH=6, for all the solutions studied. A correlation between the process efficiency and Pb(II) species in aqueous solutions, for different pH values, is presented (Figure 4.a and 4.b)

Although the solution pH changes after contacting with the biomass (the biomass having probably an amphoteric character), carrying out the process over the precipitation pH it is not justified.

Very important for the present study is to observe that similar removal efficiencies were obtained for biosorption (pH=4-5) as for the precipitation (pH=7-10).

Another important parameter of the process represents the amount of biomass added to the Pb(II) aqueous solution. Experimental data (Figure 5) show that the process efficiency is greater when the biomass concentration increases. In the mean time the sorption capacity decreases. A considerable increase of Pb(II) recoveries from the solutions it was observed for a biomass amount of 2g/L so that a supplementary addition of biomass to Pb(II) solutions does not seem to be justified from an economic point of view.

3.3 The influence of pretreatment on biomass sorption capacity

The residues of *Penicillium* were previously treated for sorption experiments development. The biomasses obtained by two different drying ways present

distinguishes sorption capacities and recovery efficiencies. Better results (Figure 1) were obtained in terms of sorption capacity for the ketone dried biomass, especially in the range of high initials concentrations.

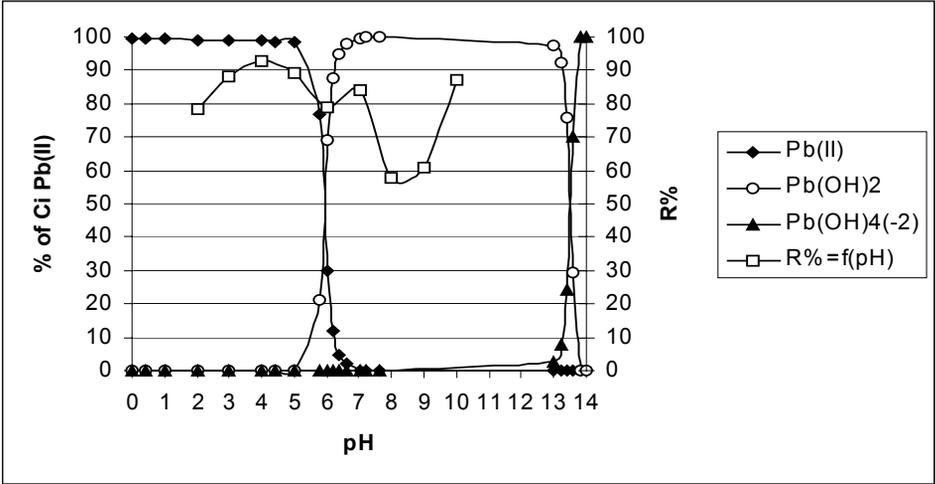


Figure 4a Correlation between R% and the Pb(II) distribution species in pH range of 0-14; $C_i \text{Pb(II)} = 5.10^{-4} \text{M}$

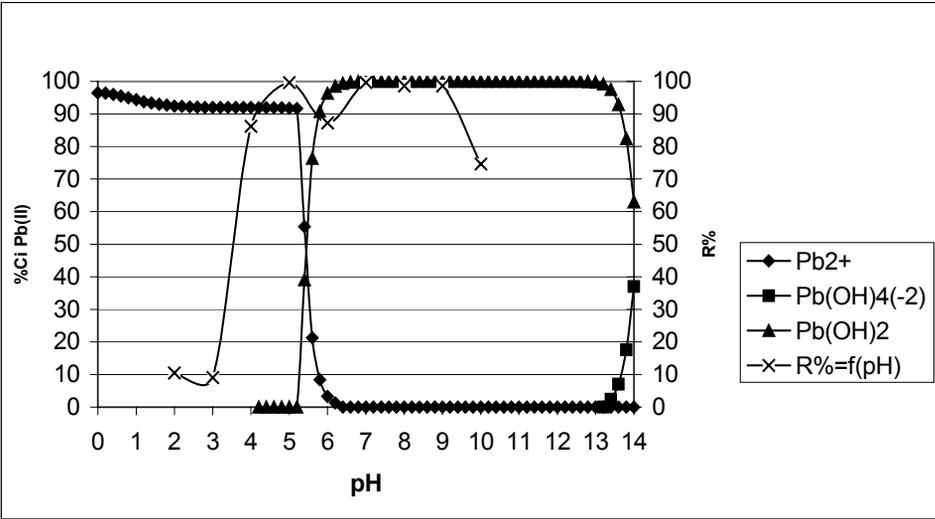


Figure 4b Correlation between R% and the Pb(II) distribution species in pH range of 0-14; $C_i \text{Pb(II)} = 5.10^{-3} \text{M}$

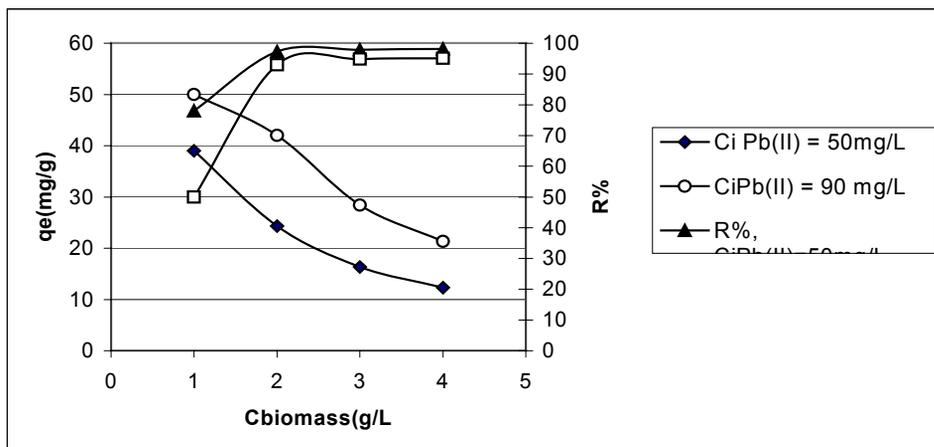


Figure 5 Influence of biomass concentration
solution pH = 4.5, biosorption time=24h, underpressure dried biomass

The IR spectra of *Penicillium* biomass before and after biosorption show the characteristic absorption bands of glycosidic OH at $760\text{-}930\text{ cm}^{-1}$, of OH from carbon hydrates at $1000\text{-}1200\text{ cm}^{-1}$, of COO⁻ from peptides at $1400\text{-}1600\text{ cm}^{-1}$, of C=O for amid bonds at 1650 cm^{-1} , of C=O from aminoacids at $1720\text{-}1750\text{ cm}^{-1}$ and of H₂O at $3000\text{-}3600\text{ cm}^{-1}$ (Table 1).

Table 1 Characteristic infrared frequencies and assignments

Sample	ν_s glic. $\alpha\text{-}1,4\text{-OH}$	ν_{as} glic. $\alpha\text{-}1,4\text{-OH}$	δ_{OH} (carbon hydrates)	ν_{COO^-} (peptide)	ν_{CO} (amide)	ν_{CO} (amino acids)	δ_{H_2O}
Biosorb. 1	758-793	917-930	1000-1200	1400-1608	1650	1724-1754	3100-3600
Biosorb. 1 +Pb	750	940	1040; 1160	1400; 1470	1650	1720-1740	3350-3450
Biosorb. 2	758-793	917-930	1000-1200	1400-1608	1650	1724-1754	3100-3600
Biosorb. 2 +Pb	750	940	1040; 1160	1400; 1470	1650	1720; 1740	3300-3400

After biosorption a new band appeared at 710 cm^{-1} corresponding to the M-O bonds and others bands disappeared or are slightly shifted (1410 cm^{-1}), corresponding to the COO⁻ groups.

The adsorption intensities for each characteristic frequency are greater for the ketone dried biomass (named biosorbent 2) comparing to the simply dried biomass (named biosorbent 1), confirming the better experimental adsorption capacities.

3.4 Biosorption-flotation parameters

As the organic sorbent is difficult to be separated from the liquid phase particularly by filtration, the separation of loaded biomass by flotation was experimented, since the biosorbent presents a natural flotation tendency. After the biosorption stage the suspensions were transferred in a flotation cell and submitted to the DAF process.

The biosorption-flotation parameters are presented in comparison with the biosorption parameters (Tables 2, 3, 4).

The dilution ratio (sample volume: water volume) was of 3:1. Pb(II) concentration and pH value were determined for the effluents. The biosorbent was the underpressure dried *Penicillium* biomass.

Table 2 %R= f(pH) dependence in Pb(II) recovery by sorption and biosorption-flotation processes; C_{biomass}=2g/L

Sorption				Sorption-flotation				
C _i (mg/l)	Sorption Time (min)	sorption pH	C _f (mg/l)	Pb(II) R(%)	pH Sorpt -flot	C _f (mg/l)	Pb(II) R(%)	Biomass R(%)
10	10	3	8,4	16	6	8,2	18	95
10	10	4	0,92	90,8	7	0,89	91,1	95
10	10	4,5	0,85	91,5	8	0,83	91,7	95
10	10	5	0,9	91	9	0,88	91,2	95
10	20	3	8,1	19	6	7,85	21,5	95
10	20	4	0,77	92,3	7	0,73	92,7	95
10	20	4,5	0,58	94,2	8	0,55	94,5	95
10	20	5	0,82	91,8	9	0,8	92	95

Table 3 %R= f(sorption time) dependence in Pb(II) recovery by sorption and biosorption-flotation processes; C_i=10mg/l; pH=4,5; C_{biomass}=2g/L

Sorption time(min)	C _f (mg/l)	R(%)	C _f (mg/l)	R(%)	Biomass R(%)
10	0,85	91,5	0,83	91,7	95
20	0,58	94,2	0,55	94,5	95
30	0,55	94,5	0,51	94,9	95

Table 4 %R= f(C_i Pb(II)) dependence in Pb(II) recovery by sorption and biosorption-flotation processes; sorption time=20 min.; pH=4,5

Sorption			Sorption-flotation		
C _i (mg/l)	C _f (mg/l)	R(%)	C _f (mg/l)	R(%)	Biomass R(%)
100	6,24	93,7	5,9	94,1	95
50	0,64	98,72	0,62	98,76	95
10	0,58	94,2	0,55	94,5	95

4 CONCLUSIONS

Biosorption–flotation may be applied for Pb(II) recovery from aqueous solutions, the efficiency being dependent on process optimal parameters. Even for more concentrated solution, the ketone pretreated biomass may ensure, in optimal work conditions, high values for Pb(II) recovery. The most important parameter of the process is the pH solution, the optimal values being in the domain of pH=4-5. For these pH values the species existing in solution are Pb(II), appropriate for biosorption, the biomass being negatively charged. The removal efficiency is closed to those characteristic for the treatments based on Pb(II) precipitation. The reduced consumption of reagent for pH adjusting and the possibility of Pb(II) recovery from the loaded biomass recommend the process as an intermediate step in wastewaters treatment. The difficulties of separating the Pb(II) loaded sorbent from the liquid phase may be avoided if DAF technique is applied. This separation process offers the possibility of higher Pb(II) removals (comparing with the simple biosorption) and also high loaded biomass removals (over 95%, the remaining being mechanical losses) so the process permits the reuse of the biomass (after regeneration) for a new cycle of biosorption-flotation. The flotation process does not require the presence of a collector.

The IR characteristic frequencies and their assignments confirm the existence of a Pb-O bond after biosorption and biosorption processes.

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Usuwanie Pb(II) z systemów wodnych poprzez biosorpcję-flotację residuum *Penicillium chrysogenum*

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Streszczenie: Środowisko wodne powstałe w wyniku eksploatacji górnictwa lub przeróbki minerałów i rud metali, posiada złożony skład chemiczny; konieczne jest zatem zastosowanie efektywnych i ekonomicznych metod jego oczyszczenia.

Ołów jest jednym z najbardziej toksycznych dla środowiska metali. Ostatnio zainteresowano się metodami separacji poprzez biosorpcję połączoną z flotacją jako metodą bardzo skuteczną. Przy użyciu residuum grzybni jako materiału sorbującego metoda ta zapewnia ekologiczną i ekonomiczną skuteczność.

Artykuł przedstawia badania dotyczące usuwania z systemów wodnych Pb(II) o różnym poziomie koncentracji ($5-1000 \text{ mgxL}^{-1}$). Badano główne parametry procesu biosorpcji: pH roztworu, stężenie Pb(II), stężenie biomasy. Flotację powietrzem zastosowano dla wydzielenia zawierającej ołów nieaktywnej biomasy z roztworu wodnego, przy czym w trakcie procesu biosorpcja-flotacja badano następujące parametry: pH, czas kontaktu sorbent-Pb(II) i koncentrację Pb(II), w celu uzyskania wysokiego poziomu separacji (powyżej 95%) obciążonej biomasy. Proces biosorpcji-flotacji umożliwia usuwanie dużych ilości Pb(II) ($R\%=85-99$).