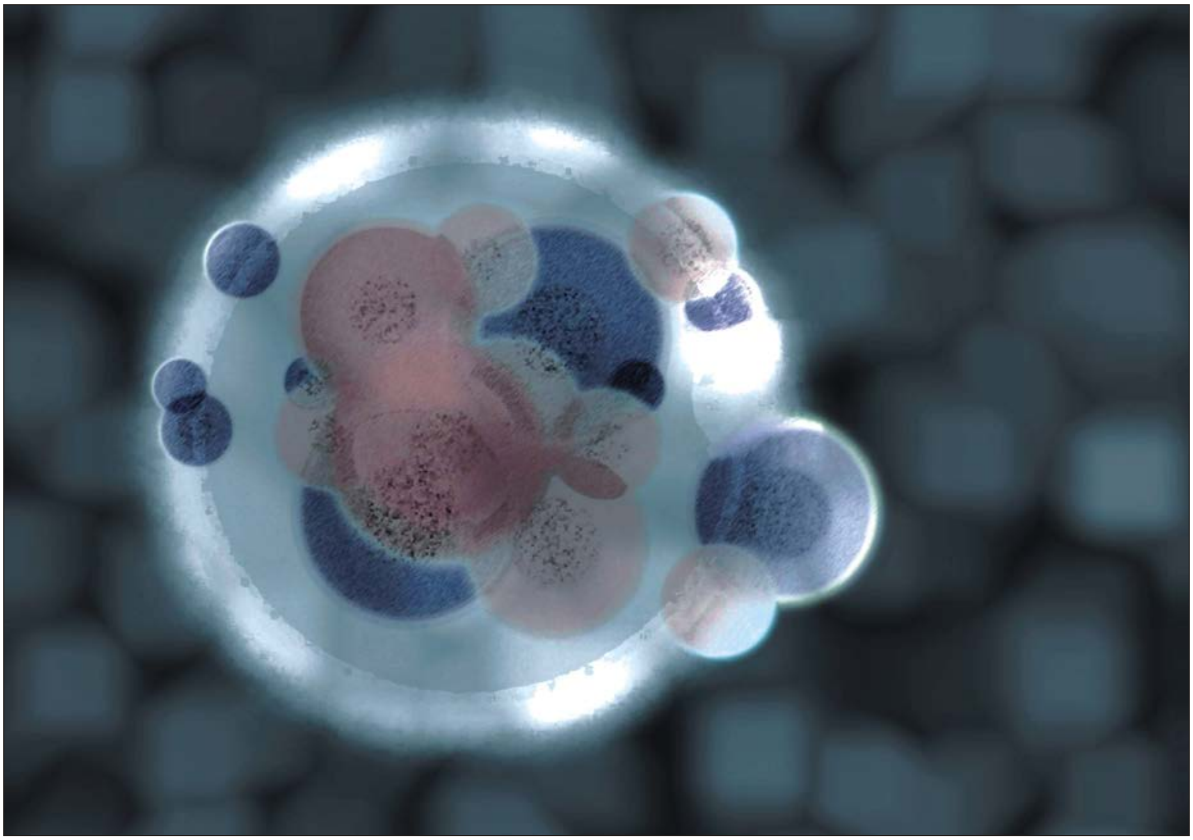

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Biosorption of Heavy Metals Using Aquatic Pycnidial and Hyptomycetes Fungi

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Abstract: This study investigates the extraction of the heavy metals (Pb, Zn, Cu and Fe) from wastes of coke Factory using aquatic pycnidial fungi, aquatic Hyphomycetes and *pencillium viridicutum*. *Cylindrocarpon heteronemum* displayed superior kinetics of dissolution of Fe, Cu and Zn compared with the other groups. Although *Pencillium viridicutum* had high activity for extraction of Pb (81%), it had low activity for dissolution of Zn and Cu metals (49 and 40%, respectively) from effluent. The effect of pH was evaluated. The results have shown that alkaline and acidity media displayed superior of extraction of heavy metals.

Key words: Aquatic pycnidial fungi, aquatic hyphomycetes, biosorption, heavy metals, industrial wastewater treatment

INTRODUCTION

Pollution by heavy metals comes from several industrial processes coke manufactures, chip manufactures, fertilizers pigments, mining and metallurgical processing (Kefalfa *et al*., 1999; Park *et al*., 1999; Kaewsam and Yu, 2001; Osman *et al*., 2006; Selatnia *et al*., 2004), therefore the role of micro-fungi in these situations is important and the search for an effective treatment technology for removal of heavy metal ions, such as Fe, Pb, Zn, Cu and Cd has included the use of micro-orgamisms (Brierly, 1991), fungi (Volesky and Holan, 1995) and algae (Chojnacka, 2003). These micro organisms were found to be capable of efficiently accumulating heavy metals (Atkinson *et al*., 1998).

Trace metals are of environmental interest both as limiting nutrients (Fe, Zn, Mn, Cu, Co, Mo and Ni) and as toxins. Toxic metals (so-called heavy metals) include Cd, Hg, Ag, Pb, Sn and Cr, although several nutrient metals, notably Zn, Cu and Ni, can also be toxic at elevated concentration (Sunda and Huntsman, 1998). Traditional technologics for the removal of heavy metal ions from solutions (ion exchange, lime precipitation, electrochemical treatment or evaporation) are often not appropriate or very expensive, particularly when metal ions are present at very law concentrations or in large solution volumes (Guibal *et al*., 1992; Fourest and Roux, 1992, Al-Saraj *et al*., 1999). Biological treatments arouse a great interest because of their lower impact on the environment with respect to chemical methods (Lodi *et al*., 1998; Chojnacka *et al*., 2004; Deveci *et al*., 2004; Selatnia *et al*., 2004).

Biosorption represents the sum of all passive interactions of the cell wall with metal ions (Hughes and Poole, 1989). These were adsorption reactions, ions exchange reactions with functional groups at the cell surface and surface complexation reactions.

Although there are many studies on biosorption and bioaccumulation processes in model systems (containing one or two contaminants), there is a lack of information on trace metal ion removal from real industrial effluents. Therefore, the main objective of this study was to evaluate trace dements removal efficiency in a real multi-component system, in order to establish operating strategies capable of achieving permissible discharge levels of trace elements in an effluent from wastes of coke Co. It is of great important not to supplement the effluent with any nutrients as this adds complexity and cost. Therefore, the main objective of this study was to evaluate trace dements removal efficiency in a real multi-component system, in order to establish operating strategies capable of achieving permissible discharge levels of trace elements in an effluent from wastes of coke Co. It is of great important not to supplement the effluent with any nutrients as this adds complexity and cost. Therefore, the microbial strain chosen should have flexible metabolism and should adjust to changing and scarce environmental conditions. These criteria are fulfilled by the aquatic pycnidial fungi (*Cystotricha striola* and *Stilbospera pistaciae*, aquatic Hyphomycetes (*Cylindrocarpon heteronemum*) and other filamentous fungi (*P. viridicutum*)

MATERIALS AND METHODS

Samples: Samples was taken from Al-Nasr Co. for coke and chemicals, Helwan, Cairo, Egypt.

This site was the plant effluent, plant sewage and mixing pools in the station of effluent treatment inside the plant. The chemical analyses of the effluent sample is shown in Table 1. It shows that the effluent sample have higher concentration of iron than other elements (Zn, Pb and Cu). Zn and Cu are trace metals, limiting nutrients and toxic.

Microorganisms and nutrient solution: Aquatic pycnidial fungi (*Cystotricha striola* and *Stilbospera pistaciae*) and aquatic Hyphomycetes (*Cylindrocarpon heteronemum*); (an isolate from twigs of cultivated and wild plant was collected from river Nile in plastic bags from the area opposite to Glucose and Starch factory (Webster and Descals, 1981).

Penicillium viridicatum which isolated from Aswan iron ore by Rehab (2004). and has the ability to solubilize phosphate and manganese from Aswan iron one without any loss from iron itself.

All strains of pycnidial, Hyphomycetes fungi and other filmentious fungi were grown/maintained on the effluent sample (1% wt/vol) prior to use as inoculum in the experiments. The growth of all the strains was conducted in an enriched salt solution containing $MgSO_4.7H_2O$ (0.4 g L^{-1}), $(NH_4)_2SO_4$ (0.2 g L^{-1}), $K_2HpO_4.3H_2O$ (0.1 g L^{-1}) and KCl (0.1 g L^{-1}), yeast extract (YE; 0.02% wt./vol) was also added to support the growth.

Extraction experiments: Extraction experiments were carried out in 250 mL Erlenmeyer flasks. Enriched salt solution (90 mL) adjusted to the required pH was transferred into each flask to which 1-2 mL of the sample was added. The flasks were them autoclaved at 1 atm and 121°C for 20 min. Following autoclaving, each flask was inoculated under aseptic conditions with a 10 mL aliquot of the selected culture producing a final volume of slurry of approximately 100 mL. To facilitate mixing of the contents and transfer of O_2 and CO_2 the flasks were shaken on the orbital shakers at room temp.

Each flask was filtrated and the filtrate was then used for analysis of metals (Zn, Fe, Pb and Cu) by atomic absorption spectrometry by the help of the Micro Analytical center faculty of science Cairo University.

Table 1: Chemical composition of the sample

Sample	Zn	Fe	Pb	Cu
	ppm			
Effluent of coke	0.146	1.767	0.183	0.07

Enriched salt solution was initially adjusted at different pH values (3, 4, 5, 7, 7.5 and 8) by using IN HCl and IN NaOH. All adjustment were carried out before sterilization of the medium by means of pH-meter.

RESULTS AND DISCUSSION

The growth of isolates in the effluent sample studied is shown in Table 2.

Not all of the 10 isolated species were able to growth and extract heavy metals (Zn, Fe, Pb and Cu). *Cystotricha striola*, *Stilbospera pistaciae*, *Cyindrocarpon heteronemum* and *Penicillium viridicutum* were the most species were grown on the samples. The mycelial dry weight of these species decreased than that of the control (media without heavy metals). This was consistent with the reports for the effect of some heavy metals on the mycelial growth of *Achlya racemosa* and *Alatospora acuminate* (El-Hissy *et al*. 1993). They were found that the mycelial dry weight of two fungal species decreased with the raise of the levels of all heavy metals salts used ($NiCl_2$, $CdCl_2$, $Pb(NO_3)_2$ and $CuSO_4$.

Complete growth inhibition of *Aspergillus flavus* occurred at 50 ppm Ni^{+2} and the *Achlya*, *Saprolegnia* sp., *C. blackeslecana* and *A. clavatus* between 50 and 100 ppm. (Babich and Stotzky, 1982, 1983). El-Sharouny *et al*. (1989) reported that all heavy metal salts ($Pb(NO_3)_2$, $NiCl_2$ and $CuSO_4$) included inhibition to

Table 2: The ability to growth of isolated fungi on liquid media with effluent sample

Species	Growth	Dry wt. g/100 mL	Dry wt. of control g mL^{-1}
Asperigillus fumigatus	-ve	-ve	0.53
A. niger	+ + +	0.03	0.66
Cladosporium macrocanpum	+	0.01	0.56
Penicillium citrinum	-ve	-ve	0.46
P. hirsutum	-ve	-ve	0.48
P. viridicutum	+ + +	0.03	0.46
P. urticae	-ve	-ve	0.40
Aquatic pycnidia			
Cystotricha striola	+ + + +	0.04	0.26
Stilbospera pistaciae	+ + + +	0.05	0.38
Aquatic Hyphomycetes			
Cylindrocarpon heteronemum	+ + +	0.03	0.35

-ve = No growth, + = weak growth, +++ = moderate growth, ++++ = strong growth

Table 3: Biosorption of heavy metals by *Cylindrocarpon heteronemum*, *Cystotricha striola*, *Stibospera pistociae* and *Pencillium viridicutum*

Species	Extraction of Heavy metals (%)			
	Zn	Fe	Pb	Cu
Cylindrocarpon heteronemum	22.0	99.9	28.0	90.0
Cystotricha striola	90.0	98.5	54.6	85.7
Stibospera pistociae	44.5	98.0	72.0	36.0
Pencillium viridicutum	49.0	97.6	81.0	40.0

mycelial growth of *Chaetomium globosum*, *Trichoderma viride*, *Fusarium solani* and *Cunninghamella echinulata* irrespective to the dose used. However, they found that *Phoma humicola*, *A. flavus*, *Pencillium chrysogenum* and *Myrothecium verrucaria* were not affected by any dose of these heavy metals.

Table 3, shows the biosorpotion of heavy metals (Zn, Fe, Pb and Cu) from the samples at neutral media (pH 7) by *C. striola*, *S. pistaciae*, *Cylidrocarpon heteronemum* and *Penicillium viridicutum*. Over 90% extraction of the iron was achieved by all the species used. However, the biosorption of lead was minimal, with 28% of lead by *Cylindrocarpon heteronemum*. This indicates the selective nature of biosorption of heavy metals from the sample.

The extraction of Zn by *Cystotricha striola* and *Cylindrocarpon heteronemum* were occurred over 80%, although was over 48% by *Penecillium viridicutum* and *Stilbospera pistaciae*. The biosorption of Zn, Fe and Cu were rapid by *Cylindrocarpon heteronemum* and *Cystotricha striola* compared with *P. viridicutum*. The findings indicated the superior biosorption capacity of aquatic pycnidial and Hyphomycetes compared with other fungi presumably due to the positive effect of elevated spore in water on the biosorption of heavy metals. Deveci *et al*. (2004) showed that the bioleaching of the complex Pb, Zn ore and concentrate using mesophilic (at 30°C), moderate (at 50°C) and extreme thermophilic (at 70°C) strains of acidophilic bacteria. They found that moderate thermophiles displayed superior kinetics of dissolution of zinc compared with the other two groups of bacteria. Dew *et al*. (1999), Konishi *et al*. (1998) Witne and Phillips (2001) and Deveci *et al*. (2004) indicated that the superior bioleaching capacity of thermophilic bacteria were effect of elevated temperature on the dissolution of sulphide minerals.

Effect of the pH value on the biosorption of heavy metals: Earlier studies on heavy metal biosorption have shown that pH is an important parameter affecting the biosorption process. Table 4 shows the effect of pH in the range of 3-8 on the biosorption rate of heavy metals (Zn) from the effluent sample by *Cylindrocarpon heteronemum*, *Cystotricha striola*, *Stilbospera pistaciae* and *Penicillium viridicutum*. The biosorption activity of Aquatic Hyphomycetes (*Cylindrocarpon heteronemum*) and pycnidial fungi (*Cystotricha striola* and *Stilbospera pistaciae*), as indicated by the dissolution rate of Zn, was decreased with high acidity and alkalinity. But at pH 7, there was an optimum dissolution rate of Zn (92 and 90%, respectively). In contrast to *P. viridicutum*, the extraction rate of zinc was observed to increase at pH 5.

These results might be indicated the inhibitory effect of increased acidity on the strain. Deveci *et al*. (2004) found that the bioleaching activity of mesophilic bacteria, as indicated by the dissolution rate of Zn was adversely affected with decreasing pH to <1.4. But, above this pH, there seemed no significant difference in the dissolution rate and extent (91-93%) of Zn, with an optimum bioleaching performance having been recorded at pH 1.8. But, the extraction rate of Zn by the extremely thermophilic was observed to increase with increasing acidity (Deveci, 2001; Porro *et al*., 1989; Arslan and Arslan, 2003; Konishi *et al*., 1998; Welham *et al*., 2000; Fenice *et al*., 2000).

The extraction of Fe by all strains was rapid, resulting in a iron biosorpiton of 98-100% in neutral and alkaline media and of 86-98% at pH 3-5 (Table 5). It should be noted that the extraction of iron was relatively low (i.e., 86% at pH 4) in comparison with those (98-100%) obtained for all strains at pH 7. The increase in the acidity to pH 3-5 led to a decrease in the oxidizing activity of all strains, indicating the inhibitory effect of increased acidity on the strains. These findings were consistent with those of Jordan (1993). The minimal precipitation of ferric iron was observed for *A. brierleyi* even at pH 1.8-2.0 probably due to the slow accumulation of ferric iron in solution coupled with the rapid reduction of ferric iron by the sulphides such as sphaleriteat 70°C. However, high temperatures would promote the precipitation of ferric iron even at low solution pH values (Arslan and Arslan, 2003; Welham *et al*., 2000) and the ability of extreme thermophiles to operate at low pH values could become important for the bioleaching process so that the formation of potentially deleterious precipitates could be minimized. Deveci et al. (2004) found that the dissolution of iron by moderately thermophilic *S. yellowstonensis* and *S. thermosulfidooxidans* at pH 1.6-2.0 was rapid.

Table 4: Effect of pH on the extraction of Zinc from the effluent sample by *Cylendrocarpon heteronemum*, *Cystotricha striola*, *Stilbospera pistaciae* and *Pencillium viridicutum*

	pH					
Species	3	4	5	7	7.5	8
Cylindrocarpon heteronemum	10	34.0	40.0	92.0	29.0	16
Cystotricha striola	74	40.0	47.0	90.0	11.0	31
Stibospera pistociae	42	39.0	31.0	90.0	44.0	30
Pencillium viridicutum	1	53.4	60.3	48.6	48.6	4

Table 5: Effect of pH on the extraction of Iron from the effluent sample by *Cylendrocarpon heteronemum*, *Cystotricha striola*, *Stilbospera pistaciae* and *Pencillium viridicutum*

	pH					
Species	3	4	5	7	7.5	8
Cylindrocarpon heteronemum	98.0	95.0	96.0	99.9	97	98.0
Cystotricha striola	90.8	86.0	96.0	98.5	98	97.6
Stibospera pistociae	89.0	86.0	96.0	98.0	97	96.0
Pencillium viridicutum	98.0	97.5	97.9	97.6	97	95.6

The dissolution of Pb was adversely affected with acidity and neutral media by all strains (Table 6). But, at pH 8 the dissolution rate of Pb was an optimum (90-100%) by aquatic pycnidial fungi (*Cystotricha striola* and *Stilbospera pistaciae* respectively). These findings were consistent with those of Feng and Aldrich (2004). The effect of alkalinity (pH 8) on the dissolution of Pb from the sample by aquatic pycnidial fungi might be led to an increase in the oxidizing activity of these strains, or the difference in the adsorption of this element on the sorbents at varied pH, indicating the positive effect of increased alkalinity on the strains. Competition between protons and metal ions for the same sites should also be considered, particularly at low pH values, as proposed by several authors (Fourest and Roux, 1992; Huang *et al*., 1991; Fourest and Volesky, 1997; Selatnia *et al*., 2004).

In contrast to the extraction rate of copper by aquatic pycnidial fungi (*Cystotricha striola* and *Stilbospera pistaciae*) was observed to increase with increasing acidity, as shown in Table 7. The extraction of Cu, was recorded to decrease by aquatic pycnidial fungi with increasing in pH between 7-8.

However, the dissolution of Cu was similar by *C. heteronemum* at pH 4 and 7 (90%). The dissolution of Cu by *P. viridicutum* was observed to increase with increasing acidity and alkalinity (77 and 74%, respectively). The biosorption of Cu and Pb on the alga was insensitive to changes in the pH in the range $5.8<pH<8.5$ (Fourest and Volesky, 1997; Feng and Aldrich, 2004). They recorded that below a pH of 4, an increase in pH resulted in an increase in biosorption of the ions. The increase in biosorption levels with an increase in pH can be explained by the influence of the number of negative surface charges, which depends on the dissociation of functional groups. In addition, this could partly explain the low concentrations of Cu and Pb retained by the biosorbent at pH values below 4, since most functional groups are expected to become dissociated at neutral pH values only.

Table 6: Effect of pH on the extraction of Lead from the effluent sample by *Cylendrocarpon heteronemum*, *Cystotricha striola*, *Stilbospera pistaciae* and *Pencillium viridicutum*

	pH					
Species	3	4	5	7	7.5	8
Cylindrocarpon heteronemum	34	81.0	61.0	28	92	80.0
Cystotricha striola	48	1.0	87.0	55	55	100.0
Stibospera pistociae	40	48.0	88.0	72	78	90.0
Pencillium viridicutum	66	57.9	80.3	81	71	62.8

Table 7: Effect of pH on the extraction of Cu from the effluent sample by *Cylendrocarpon heteronemum*, *Cystotricha striola*, *Stilbospera pistaciae* and *Pencillium viridicutum*

	pH					
Species	3	4	5	7	7.5	8
Cylindrocarpon heteronemum	75.7	90	70	90	70	67
Cystotricha striola	68.5	81	91.4	85.7	85.7	82.9
Stibospera pistociae	55.5	80	90	36	40	40
Pencillium viridicutum	78	77	72.8	40	40	74

In the present study, microorganisms are very useful agents in removal heavy metals by biosorption from a sample industrial effluent. There is lack of the data on the behaviour of microorganisms in industrial effluents. The present investigation showed that Aquatic pycnidial fungi and Aquatic Hyphomycetes were capable to biosorption heavy metals (Zn, Fe and Cu) from effluent sample. However, *P. viridicutum* had high capacity for biosorption of Pb from effluent. It was found that Aquatic pycnidial fungi and Hyphomycetes (*Cylendrocarpon heteronemum*) were capable of the selective removal of trace elements from the effluent of coke factory, which contained elevated levels of heavy metals.

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