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Biosorption of Zinc from Effluent of Rubber Product Manufacturing

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Zinc removal from effluent of rubber product manufacturing through biosorption by four microbial species (Zoogloea ramigera, Aerobacter aerogenes, *floc-forming bacteria and floc-forming yeast) was studied. Growth kinetics and exopolymer production by the species cultured on synthetic media were obtained. Studies conducted using rubber effluent with initial zinc concentration of 29 mg/L showed that the initial zinc uptake rates and equilibrium adsorption capacities achieved by the microorganisms ranged from 18.3 to 42 3 mg Zn/g biomass·min and 88 to 206 mg Zn/g biomass, respectively.* A. aerogenes *gave the highest initial zinc uptake rate and equilibrium adsorption capacity, followed by the floc-forming yeast. Further investigation on the floc-forming yeast showed that the optimum pH for zinc adsorption by the species was 6.5.*

Effluent from rubber product manufacturing contains hazardous chemicals including heavy metals derived from chemicals used in latex preservation and compounding processes. Based on a recent survey[1], zinc has been identified as the main heavy metal component with concentrations reaching 32 mg/L. Magnesium, iron, copper, nickel and manganese have also been detected but at lower concentrations (below 2 mg/L). The effluent has to be treated to comply with the Malaysian Standards for Watercourse Discharge of Industrial and Sewage Effluents (1979)[2]. The standard requires the concentration of zinc in the final discharge to be below 1 mg/L.

Removal of heavy metals from industrial effluent has been limited to the physico-chemical processes such as chemical precipitation, ion exchange, membrane filtration and reverse osmosis[3,4]. The survey[1] showed that chemical precipitation was widely used for pre-treating the rubber effluent. This process generated a large amount of hazardous chemical sludge which is considered a scheduled waste. The sludge is usually dewatered and stored prior to disposal. The accumulating amount of dewatered sludge requires a large storage area, resulting in a major constraint since most factories are located in limited land areas.

The treatment and disposal of scheduled wastes from the rubber industry has been regulated by the government of Malaysia. The wastes must be collected and transported to a

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central treatment facility for final disposal The current treatment fee is RM2790/tonne of sludge, excluding the costs of collection and transportation This high cost is a major drawback for Malaysian rubber industry which is already facing stiff price competition from other natural rubber producing countries Thus, an alternative cost-effective method of removing heavy metals that can reduce the amount of hazardous sludge production is required

With recent advances in the field of biotechnology, biological methods could possibly be more effective than the chemical precipitation for the removal of heavy metals from industrial effluents Removal, concentration and recovery of certain heavy metals by microbial systems have been reported[5–8] Heavy metals removal by microbial systems have been observed to take place in association with extracellular products (exopolymers), within cell membrane matrix and intracellularly Mechanisms of heavy metal removal consist of physical-chemical adsorption, complexation, precipitation and active uptake *Z ramigera*, a dominant species in activated sludge process was shown to adsorb copper efficiently up to 0 34 g Cu/g cells dry weight[9] Level of metal accumulation by various microbial species was reported to vary from 0 08 to 0 57 g metal/g cells dry weight[10] Waste microbial biomass from fermentation industry could be used as a cheap adsorbent to reduce the cost of heavy metal removal[11]

An investigation was carried out to study the potential of using a biological system for removing zinc from effluent of rubber product manufacturing This paper discusses the results of this investigation involving the use of four exopolymer producing microbial species for the removal of zinc from the effluent of rubber product manufacturing

MATERIALS AND METHODS

Microbial Growth and Exopolymer Production

Three bacterial species and a floc-forming yeast (FFY) were studied for biomass and exopolymer production The bacterial species were *Aerobacter aerogenes* IFO 12059, *Zoogloea ramigera* ATCC 25935 and a floc-forming bacteria (FFB) isolated from sludge of an oxidation ditch treating rubber processing effluent *Z ramigera*, a common bacterium in an activated sludge system, and *A aerogenes* were chosen as both species have been shown to produce exopolymers when cultured in media containing carbon source in excess (C N ratio greater than 38)[12]

Exopolymer production studies of the microbial species were carried out by culturing these species in a 2-L fermenter agitated at 200 r p m – 250 r p m and aerated by filtered air at 4–6 cm^3/min for a period of 160 h *Table 1* shows the composition of culture media for the species which were prepared in distilled water at pH 7 0

Exopolymers produced by these bacteria were extracted and quantified according to the method described by Gasdorf *et al*[13] Exopolymers from the viscous culture were extracted by centrifuging the culture medium at 10 000 r p m (17 000 g) for 30 min The supernatant was decanted and the exopolymers in the supernatant were precipitated by adding 1 g/L KCl per 100 ml solution and 2 volumes of ethanol The entire precipitate was removed and redissolved in a volume equal to the original

TABLE 1 COMPOSITION OF GROWTH MEDIA

Constituents	Concentration (g/L)			
	A aerogenes	*Z ramigera*	FFB	FFY (*Medium A*)
Glucose	10 0	25 0	25 0	30 0
$(NH_4)_2SO_4$	0 3	1 0	1 0	2 0
K_2HPO_4	2 0	2 0	2 0	4 0
KH_2PO_4	1 0	1 0	1 0	–
$MgSO_4$ $7H_2O$	0 4	0 2	–	0 8
CaCl $6H_2O$	0 01	–	–	–
$MnSO_4$ $4H_2O$	0 005	–	–	0 05
Yeast extract	–	0 001	0 01	–

volume taken The dissolved exopolymer was then reprecipitated as previously carried out and subsequently air dried in pre-weighed crucibles at 70°C for 24 h The dry weight of microbial cells was determined by centrifuging 20 ml of cells at 8 000 r p m (14 000 g) for 30 min and drying the settled cells after a series of washing in pre-weighed crucibles at 100°C for 24 h

Effluent Characteristics

Effluent used in this study was obtained from a rubber product manufacturing factory The effluent was acidic with a pH of 3 69 The pollutional strength of the effluent as determined by the chemical oxygen demand (COD) was 1064 mg/L The effluent contained 85 mg/L suspended solids (SS) and 55 mg/L volatile suspended solid (VSS)

Zinc Uptake by Micro-organisms

Zinc removal study was carried out by mixing 50 ml of the microbial cultures obtained after 160 h of fermentation with 500 ml of diluted rubber effluent with an initial zinc concentration of 29 mg/L Prior to mixing, pH of the effluent was adjusted to 7 0 with dilute NaOH solution and then filtered to remove the suspended solids

Mixture of the microbial culture and effluent was stirred for a period of 3 h to provide sufficient time to reach adsorption equilibrium The mixture was stirred at 60 r p m for 5 min followed by slow stirring at 20 r p m for up to 3 h at 25°C Samples were taken at 5, 30, 60, 120 and 180 min over the stirring period The samples were centrifuged at 8000 r p m (14 000 g) for 15 min Both the settled biomass and supernatant portions were sent for zinc analysis using atomic absorption spectrophotometry (AAS)(Unicam Model 939, UK) with a detection limit of 0 1 mg/L The experiment was conducted in triplicates for each microbial species

Further Investigation on Optimum pH of the Floc-forming Yeast

Results of the initial evaluation showed that the FFY possesses suitable physical characteristics and adsorption kinetics for large scale applications. The FFY was therefore selected for further investigation on the influence of effluent pH on the zinc adsorption capacity of the species.

Biomass production was repeated by culturing the FFY in 1.5 L *Medium A* in a 2 L fermenter agitated at 250 r.p.m. and aerated at 4–6 cm^3/min. The cells were harvested by centrifugation after 160 h of culturing. The untreated cells were dried at 70°C for 24 h for subsequent biosorption study. FFY cell suspension was prepared by suspending 0.5 g of the dried cells in 500 ml distilled water to give a dry cell concentration of 1 g/L. The suspension was then homogenised in a mixer to break cell aggregates.

The zinc uptake study was carried out by adding 20 ml of the homogenised cell solution to 250 ml of the effluent after initial pH adjustment with 1 M NaOH solution. The mixture was stirred at 60 r.p.m. for 5 min followed by slow stirring at 20 r.p.m. for 3 h at 25°C. Samples were taken at 5, 30, 60, 120 and 180 min over the mixing period. Samples were centrifuged at 8000 r.p.m. (14 000 g) for 15 min and the supernatants were analysed for zinc using AAS.

RESULTS AND DISCUSSION

Microbial Growth and Exopolymer Production

The species selected for this investigation were cultured in media with excess glucose as the carbon source. Growth kinetics and exopolymer production by the four microbial species are summarised in *Table 2*. The FFY achieved the highest growth rate of 0.058 h^{-1} and the highest cell concentration of 6.41 g/L was obtained by *Z. ramigera*.

Production of exopolymers by the species was shown to be growth associated. The highest exopolymer concentration of 1.73 g/L was produced by the FFB after 160 h of culturing with a yield factor of 0.01 g exopolymer/glucose. Microscopic examination of the cultures revealed a gelatinous matrix form of exopolymers produced by *Z. ramigera* and *A. aerogenes* resulting in viscous broth with uniform cell distribution.

Both FFY and FFB produced a capsulated form of exopolymer with floc characteristics. The FFY showed a distinctive cell flocs beginning at the stationary phase of growth. At the end of the culture period, the size of the FFY flocs ranged from 1 mm to 5 mm in diameter and readily settled in the culture broth while the FFB showed relatively tiny flocs with size less than 1 mm.

Zinc Adsorption by Micro-organisms

Many micro-organisms maintain an ability to form heavy metal complexes with the presence of acidic polysaccharides on cell surfaces which provide amino, carboxyl, phosphate and sulphate binding groups. The presence of hydroxyl groups and an overall negative charge of the extracellular polysaccharides (exopolymers) are important factors in heavy metal binding. *Z. ramigera* has been known to form a gelatinous matrix of negatively charged extracellular polysaccharides around the cells for the attachment of ions[5].

TABLE 2. GROWTH KINETICS AND EXOPOLYMER PRODUCTION

Species	Maximum cell concentration (g/L)	Exopolymer concentration (g/L)	Specific growth rate (h^{-1})	Yield factor Yp/s[a]
A. aerogenes	1.54	1.44	0.003	0.07
Z. ramigera	6.41	1.28	0.017	0.03
FFB	3.44	1.73	0.018	0.01
FFY	3.52	1.21	0.058	0.06

[a]Unit for Yp/s is g exopolymer/g glucose

Extensive amount of information has been gathered on the uptake of heavy metals by micro-organisms from research carried out using synthetic effluents containing single metal ions. Relatively little is known about the metal uptake in actual industrial effluents. Actual effluents are normally a complex mixture of metal ions and other components such as organic compounds and suspended solids[1].

In this investigation, actual effluent from a rubber product manufacturing factory was used. The characteristics of this effluent were given in the previous section. The presence of various components in actual effluents give rise to interactive effects which influence the ability of micro-organisms to adsorb metal ions. Mixture of different ions in synthetic effluent has been shown to influence ion uptake and types of behaviour displayed by micro-organisms[14]

The results of zinc adsorption by micro-organisms were presented as percentage removal (%), adsorbed zinc per unit of biomass (mg Zn/g biomass) and initial adsorption rate (mg Zn/g biomass·min), calculated from the plot of adsorbed zinc per unit biomass *versus* time (min). *Figure 1* to *4* show the plots of zinc adsorption by the species investigated with the initial zinc concentration of 29 mg/L at pH 7.0.

The percentage zinc removal at equilibrium ranged from 72% to 96% with the highest removal achieved by *Z. ramigera* at 96% removal. *Z. ramigera* showed the highest percentage of zinc removal due to higher cell concentration in the culture broth mixed with the effluent. In all experimental runs, the volume of the culture broth mixed with the effluent was constant but cell concentration varied

All species investigated showed similar pattern of adsorption with initial rapid uptake for the first 5 min followed by slow uptake for the remaining of the mixing period The overall pattern showed that the initial 5 min was crucial for zinc adsorption rates. *A. aerogenes* showed the highest initial adsorption rate of 42.3 mg Zn/g biomass·min. The FFY, FFB and *Z. ramigera* gave lower initial adsorption rates of 27.7, 22.9 and 18.3 mg Zn/g biomass·min, respectively (*Figure 5*).

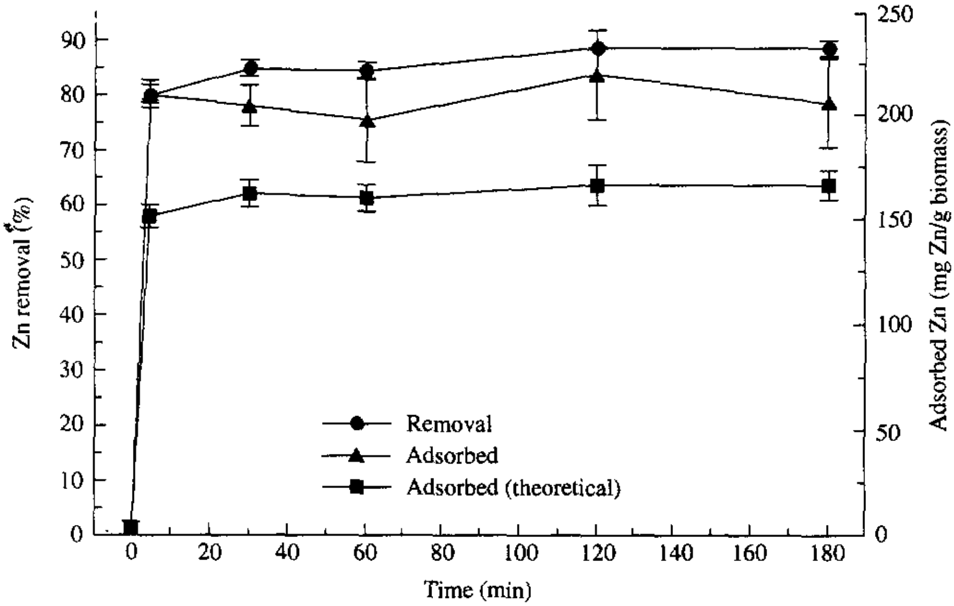

Figure 1. Zinc biosorption by fresh biomass of Aerobacter aerogenes *(pH of effluent: 7.0; data are presented as mean ± s.d.; n=3).*

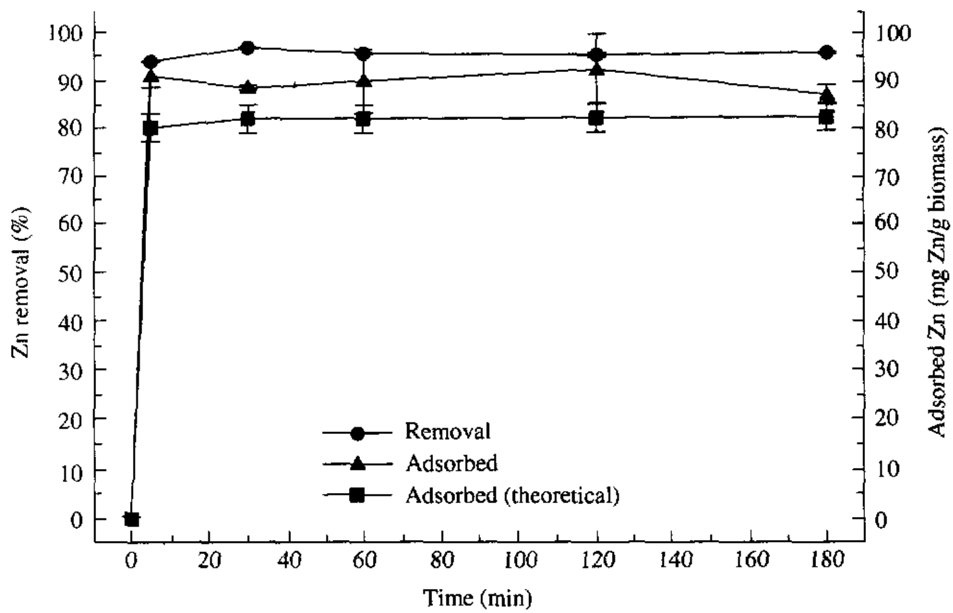

Figure 2. Zinc biosorption by fresh biomass of Zoogloea ramigera *(pH of effluent: 7.0; data are presented as mean ± s.d.; n=3).*

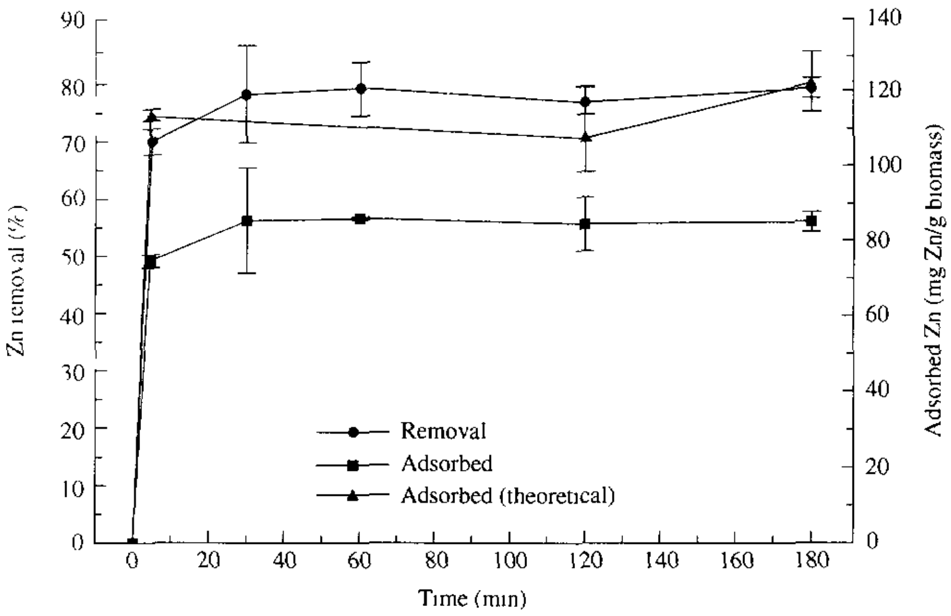

Figure 3 Zinc biosorption by fresh biomass of the floc-forming bacteria (FFB) (pH of effluent 7 0 data are presented as mean ± s d , n=3)

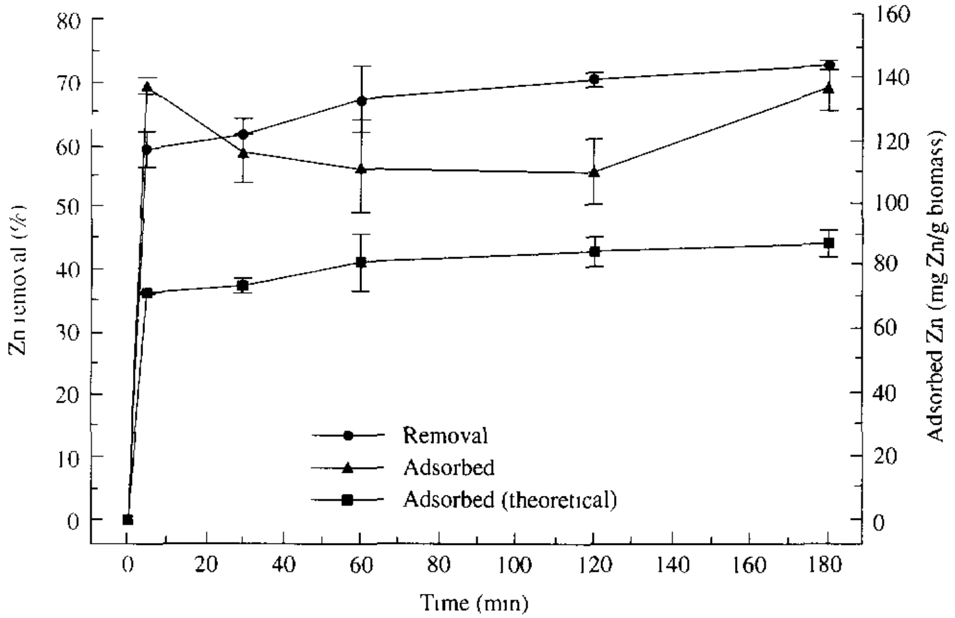

Figure 4 Zinc biosorption by fresh biomass of the floc-forming yeast (FFY) (pH of effluent 7 0, data are presented as mean ± s d , n=3)

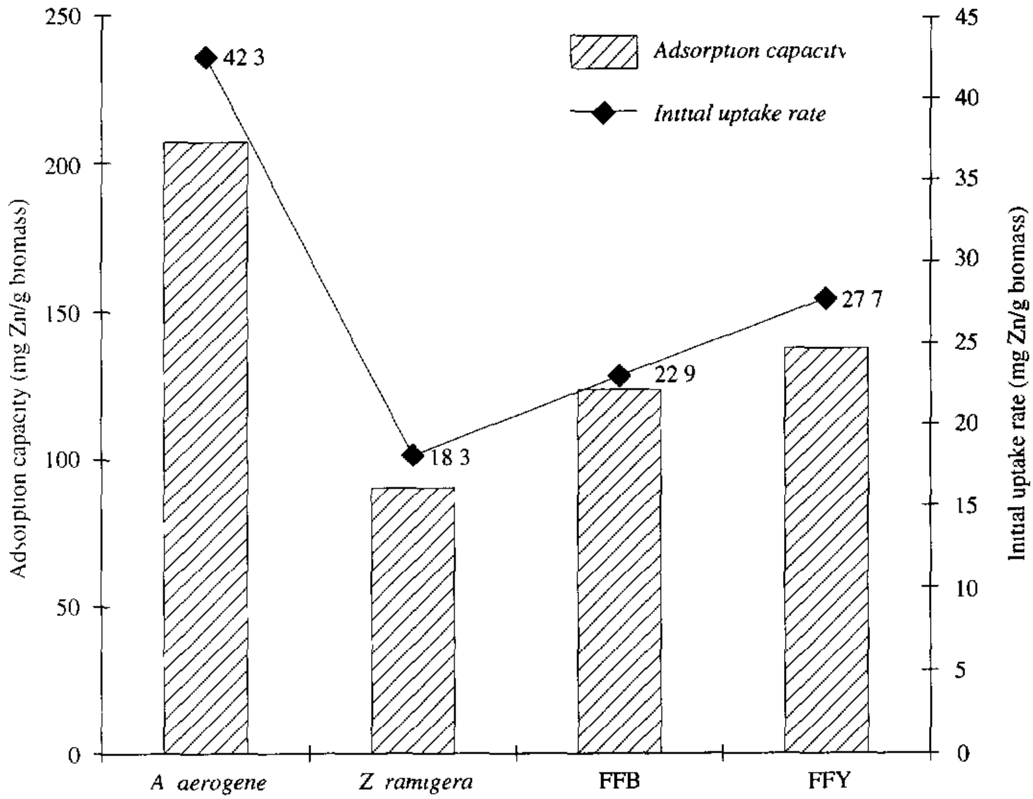

Figure 5 Summary of zinc adsorption kinetics by the all microbial species investigated (data are presented as average of 3 experiments)

Similar adsorption pattern and kinetics were reported, involving adsorption of lead (II) and copper by *Z ramigera* in synthetic effluents[5 9] The uptake of metal ions by micro-organisms in a batch system involves two stages The first stage is an initial rapid uptake (passive uptake) and the second stage is a slower process (active uptake)[15] The results show that adsorption is the main process contributing to metal uptake by micro-organisms Adsorption involves a non-directed physical process with chemical complexation between metal ions, components of cell surface and exopolymers

The equilibrium adsorption was obtained after approximately 30 min of the initial rapid uptake period in all species investigated *A aerogenes* obtained the highest equilibrium zinc adsorption capacity of 206 mg Zn/g biomass This was followed by the FFY, FFB and *Z ramigera* at 137, 123 and 88 mg Zn/g biomass min, respectively The variation in adsorption capacity of zinc by the species indicates that the ability to accumulate metal ions varies among micro-organisms This is attributed to the specific composition of exopolymers and

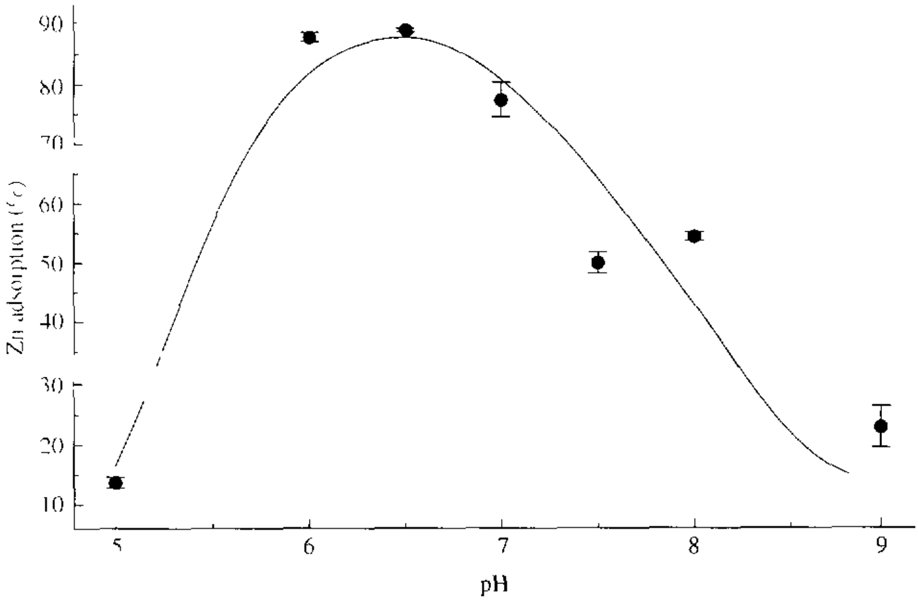

Figure 6 The effect of initial pH of effluent on the zinc adsorption by dry biomass of the floc-forming yeast (FFY) (concentration of biomass 1.0 g/L, data are presented as mean ± s.d., n=3)

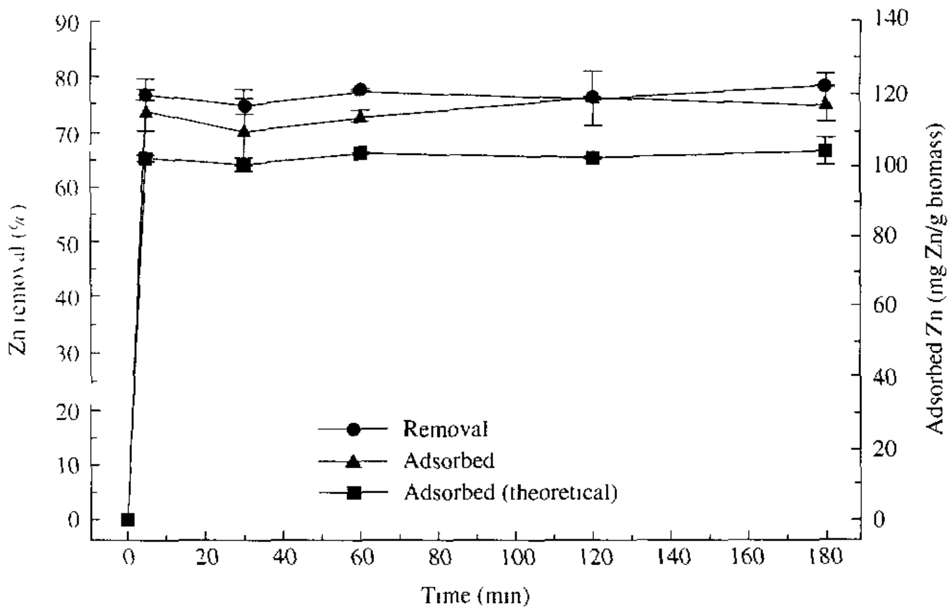

Figure 7 Zinc biosorption by dry biomass of the floc-forming yeast (FFY) (pH of effluent 7.0, concentration of biomass 1.0 g/L, data are presented as mean ± s.d., n=3)

cell wall components that determine the metal binding properties of microbial species.

In *Figures 1 to 4*, a plot was included to represent the theoretical adsorption that was calculated using a mass balance based on the remaining zinc concentration. This was done to verify the transfer of zinc ion from the effluent to the biomass. All species investigated showed lower theoretical adsorption than the actual adsorption. The closest agreement was shown by *Z. ramigera* with the average difference between the theoretical and actual adsorptions ranging from 5% to 16%.

In principle, the amount of zinc removed from the solution should be equal to the amount of zinc adsorbed by the biomass resulting in an identical plot for both the theoretical and actual adsorptions. It was assumed that the higher value of actual adsorption was due to the condition of the biomass on adding to the effluent. In the present study, the biomass was added to the effluent in its original culture broth without washing. Some fermentation products adhered to the microbial cells could have assisted in the uptake of zinc by the biomass by providing extra adsorptive sites. Based on this assumption, the dried and unwashed biomass was chosen to be used for further investigation.

Optimum pH for the Floc-forming Yeast

The FFY has been shown to produce firm cell flocs of suitable sizes which withstood shear rate (60 r.p.m.) and able to maintain an aggregated form throughout the mixing period. This characteristic is favourable for large scale applications as it can assist in the separation of metal-laden biomass from effluent. It also avoids the need for carrier materials to immobilise cells. There is also a possibility that the microbial floc can be repeatedly used. The FFY was selected for further investigation based on the favourable characteristic and adsorption kinetics observed in the preliminary investigation.

The pH of the adsorption media influences the biosorption mechanisms of metal ions by micro-organisms[5,10]. The pH probably affects the interaction between metal ions in solution and the adsorptive sites of biomass. The optimum pH for metal uptake by micro-organisms varies probably due to the variability in the effect of the pH on the conditions of the adsorptive sites of the micro-organisms.

Figure 6 shows that the uptake of zinc ions was sensitive to changes in pH of the effluent. Zinc adsorptions above 70% were achieved between pH 6.0 and pH 7.0. The optimum pH for the FFY was found to be 6.5 with 88% adsorption. It is postulated that at pH around 6.5, which is above the isoelectric point, there is a net negative charge on the cells and the ionic state of ligands such as amino and carboxyl groups will promote reaction with zinc ions. This is enhanced by the amphoteric behaviour of zinc hydroxides which is less soluble in near neutral solutions.

Zinc adsorption by the dry biomass of the FFY at pH 7.0 is shown in *Figure 7*. Comparing *Figure 7* with *Figure 4*, it can be seen that the initial zinc uptake rate and adsorption capacity of the dry biomass were lower than those of the fresh biomass (*Figure 4*). However, the dry biomass showed better agreement between the actual and theoretical adsorption capacities as shown in *Figure 7*. This could be due to the absence of fermentation products on the dried biomass because of pre-washing. It was earlier assumed that the fermentation products adhered to the wet biomass were responsible for the additional uptake of zinc by the biomass

CONCLUSION

The FFY was found suitable as an adsorbent for removing zinc from rubber product manufacturing effluent, based on its favourable adsorption kinetics and physical characteristics However, in selecting a microbial species for removing zinc from effluent for large scale applications, consideration should not be based solely on the adsorption and growth kinetics Other factors, including the physical characteristics of biomass, physico-chemical influence of the process conditions, production of biomass and modes of operation, should also be considered

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