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BIOSORPTION OF Mn (II), Co (II) and Cr (VI) IN A HORIZONTAL ROTATING TUBULAR BIOREACTOR: EXPERIMENTS AND EVALUATION OF THE INTEGRAL BIOPROCESS MODEL

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Abstract - In this research, a multi heavy metals removal process with mixed microbial culture was examined in a horizontal rotating tubular bioreactor (HRTB) with different combinations of process parameters. Three metals were selected as examples of cations (manganese and cobalt) and oxy-anion (hexavalent chromium). Hydrodynamic conditions and biomass sorption capacity in the HRTB had the main impact on the heavy metals removal efficiencies, which were for Mn^{2+} 87.0-93.6%, Co^{2+} 89.0-95.7% and Cr^{6+} 99.7-100%, respectively. For the bioprocess description in the HRTB, the integral bioprocess model that combines hydrodynamics, mass transfer and kinetics was used. This model was evaluated for the new experimental conditions and average variances between experimental and simulated data were in the range of 0.12 - $3.21 \cdot 10^{-3}$. The results obtained clearly show that the integral bioprocess model is able to describe the heavy metal removal process in the HRTB.
Keywords: Heavy metals removal; Mixed microbial culture; Biosorption; Integral bioprocess model; Horizontal rotating tubular bioreactor.

INTRODUCTION

Wastewaters polluted with heavy metals have always been a very serious problem due to the fact that these elements are not biodegradable and they can accumulate in living tissues, causing serious health problems. Many industrial processes are closely related to environmental pollution with heavy metals. Therefore, there is a constantly growing concern over the effect of heavy metals on humans and aquatic ecosystems. The Agency for Toxic Substances and Disease Registry classifies heavy metals as human carcinogens and health risk substances (Valko *et al*., 2005; Deng *et al*., 2006).

There are many conventional methods (physical and chemical) for heavy metals removal, but in general they generate environmentally hazardous chemical by-products that result in waste that is hard to

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treat. In addition, commonly used processes such as ion exchange or membrane technology are quite expensive methods for large wastewater quantities that usually contain low concentrations of heavy metals (Lesmana *et al.*, 2009; Wang and Chen, 2009; Gayathri and Senthil Kumar, 2010; Barakat, 2011).

As an alternative, different biological methods could be applied because they do not destroy metals, but concentrate and immobilize them (Gavrilescu, 2004; Vieira *et al*., 2012; Michalak *et al*., 2013; Sicupira *et al*., 2014; Vieira *et al*., 2014). Biosorption is the removal of metals and their complexes by biological materials such as active or inactive microorganisms, microbial aggregates or biofilms (Singh *et al*., 2006; Baysal, *et al*., 2009; Xie, *et al*., 2013). These materials can efficiently remove heavy metals from solutions and therefore they are ideal adsorptive media for wastewaters with low metal ion concentrations. Microbial metal accumulation has received much attention during recent years, due to the potential use of microorganisms for treatment of metal-polluted water or wastewater streams (Gadd, 2008). Several bacterial species have been identified that remove toxic heavy metals (Singh *et al*., 2006; Yilmaz *et al*., 2010). For biosorption of heavy metals microbial biofilms show great potential in wastewater treatment systems. Therefore, different bioreactor types (e.g., trickling filters, fluidized or packed bed bioreactors, thin layers or biodisc reactors) were developed for different wastewater treatment systems (Gavril and Macoveanu, 2000; Nicolella *et al*., 2000; Chojnacka, 2010; Chu, 2010).

The horizontal rotating tubular bioreactor (HRTB) was designed as a combination of a thin layer (Moser, 1985) and a biodisc reactor (Kargi and Eker, 2001). Its interior is characterized by O-ring shaped partition walls that provide additional area for biofilm formation. Major advantages of the HRTB use in wastewater treatment are higher bioprocess efficiency and stability compared to the bioreactors with suspended microbial cells. Higher bioprocess efficiency is a consequence of plug flow conditions in the HRTB that are related to the formation of concentration gradients along the bioreactor length and therefore inhibition and/or repression bioprocess kinetics can be avoided. Due to the biofilm formation on the inner surfaces of the HRTB biomass washout happens very rarely even when relatively high inflow rates are used. Biofilm thickness in the HRTB can be controlled by bioreactor rotation speed and consequently substrate and/or oxygen limitation in the biofilm can be prevented. Furthermore, tubular bioreactors (as well as the HRTB) are characterized by relatively low operational energy demands.

Due to the great potential of the HRTB, different bioprocesses were studied in this bioreactor such are anaerobic (Ivančić *et al*., 2004), aerobic (Slavica *et al*., 2004) as well as a combination of aerobic and anaerobic bioprocesses (Rezić *et al*., 2007). Furthermore, heavy metals removal (Fe^{2+}, Cu^{2+}, Ni^{2+} and Zn^{2+}) with mixed microbial culture was also studied in the HRTB (Rezić *et al*., 2011a) and the integral bioprocess model was established that combines a mixing model and a metal ion diffusion-adsorption model (Rezić *et al*., 2011b). In recent years, many mathematical models have been established to represent the biosorption of different metal ions in different bioreactor systems (Lesmana *et al.*, 2009; Ong *et al*., 2013). The hydrodynamic behavior of these bioreactor systems was mostly described by a dispersion model. For description of the biosorption process in these systems different equations can be used such are Bohart-Adams (Wang, 2007; Kargi and Cikla, 2007; Chu, 2010; Chatterje and Schiewer, 2011), Langmuir (Chen and da Silva *et al*., 2002; Kogej *et al*., 2010; Ghasemi *et al*., 2011), Freundlich (Kogej and Pavko, 2001; Quintales *et al*., 2009a; Rezić *et al*., 2011a,b), a pseudo-second-order rate expression (Martins *et al*., 2014), or Yoon-Nelson (Chatterje and Schiewer, 2011).

The novelty of this research is the study of the harmfulness of two less toxic metals (Co^{2+} and Mn^{2+}) and one more toxic metal (Cr^{6+}) from textile wastewater on the mixed microbial culture in the HRTB in order to check the bioprocess stability and repeatability. Furthermore, in this study for bioprocess monitoring in the HRTB a more efficient and sensitive analytical method (ICP-MS) was used. Bioprocess performance in the HRTB was described by the integral bioprocess model that combines mixing and diffusion-adsorption models (Rezić *et al*., 2011b) in order to confirm its ability to describe different heavy metals removal processes in the HRTB, as well as to prove its potential for the use in the scale-up procedure.

MATERIALS AND METHODS

Mixed microbial culture was isolated from ground soil samples located inside iron, vinyl and cement factories area (industrial area located near the town of Split, Croatia). Soil samples contain 4 - 35 times higher heavy metals concentrations than unpolluted soil (Lovrenčić *et al*., 2005). Therefore, it is expected that microbial cultures present in these soil samples are capable of growth in the medium with

increased concentrations of heavy metals (Kavamura and Esposito, 2010). Synthetic liquid medium was used for isolation of mixed microbial culture from soils samples that contained the following ingredients (g L^{-1}): glucose, 10; $FeSO_4 \cdot 7H_2O$, 0.62; $CuSO_4 \cdot 5H_2O$, 0.49; $NiSO_4 \cdot 6H_2O$, 0.56; $ZnSO_4 \cdot 7H_2O$, 0.55; $CoCl_2 \cdot 6H_2O$, 0.50; $MnSO_4 \cdot H_2O$, 0.39; $K_2Cr_2O_7$, 0.20; Pb-acetate$\cdot 3H_2O$, 0.23; yeast extract, 0.3; and tripton, 0.3 (pH = 4.40). The isolation procedure started by the addition of 5 g of soil samples into 200 mL of sterile synthetic medium and cultivation on the rotary shaker at 30 °C /48 h (rotation speed 120 min^{-1}). After that, the obtained mixed microbial culture was plated on the solid synthetic medium (synthetic liquid medium for isolation with 20 g L^{-1} of agar) at 30 °C /48 h. Dominant microbial species were selected and cultivated again several times (in liquid synthetic medium as well as plated on solid synthetic medium) in order to obtain the most active microbial species.

In this research, the isolated mixed microbial culture was cultivated at room temperature (20 ± 1 °C) in a synthetic medium containing (g L^{-1}): glucose, 10; $MnSO_4 \cdot H_2O$, 0.39; $CoCl_2 \cdot 6H_2O$, 0.51; $K_2Cr_2O_7$, 0.20; yeast extract, 0.3; and tripton, 0.3 (pH = 4.2-4.6). In this investigation, media were sterilized at 121 °C for 20 minutes.

The HRTB was constructed as a 2.0 m long stainless steel tube with an inside diameter of 0.25 m. The interior of the HRTB was divided by O-ring shaped partition walls with an inside diameter of 0.19 m. The distance between the partition walls was 0.02 m and the liquid volume in the HRTB was 15 liters. The HRTB was horizontally placed on bearings that enable rotation of the whole bioreactor. The aeration was done through the central tube fixed on the axis of the HRTB. The aeration tube was submerged in the medium at five positions along the bioreactor to improve the aeration efficiency. During the entire research airflow rate was constant (152 L h^{-1}). Systems for sampling of broth and biofilm were placed along the bioreactor at 0.40 m intervals. The device for biofilm thickness determination was made of a flat plate (dimensions 0.02 x 0.02 m) fixed on the cover of the sampling place. The required amount of suspended microbial biomass (7.5 L) for the inoculation of the HRTB was obtained by batch cultivation in the stirred tank bioreactor. After 24 hours the feeding process was started at a rate of 1 L h^{-1} and bioreactor rotation speed of 10 min^{-1}. Mixed microbial culture initially grew in suspension and then a biofilm developed gradually on the bioreactor inner surfaces. It took 15 days (24 medium residence times) from the inoculation to obtain a stable biofilm in the HRTB and then it was considered as ready to conduct performance studies with different combinations of process parameters: medium inflow rate (0.5, 1.0 and 2.0 L h^{-1}) and bioreactor rotation speed (5, 15 and 30 min^{-1}). During this beginning period, the biofilm thickness measurement was also performed (every 12 hours) as well as monitoring the biofilm erosion process in order to evaluate biofilm stability in the HRTB. In this research, the inlet glucose concentration in the medium was constant (10 g L^{-1}), as well as the inlet multi-metal ion concentrations (Co^{2+} = 0.125 g L^{-1}, Mn^{2+} = 0.125 g L^{-1} and Cr^{6+} = 0.125 g L^{-1}), respectively. The dynamics of the bioprocess in the HRTB was monitored by withdrawing the samples along the bioreactor length after five residence times from when the new set of process parameters was established. In this research, for each combination of bioreactor process parameters (*n* and *F*), samples were withdrawn in triplicate (in total, at least 99 samples) at time intervals of every 12 hours after the new set of HRTB process parameters was established.

The biomass concentration in suspension was determined by centrifugation of 35 mL samples for 20 min at 4500 min^{-1} (3629 g), washing twice with demineralised water and then drying at 105 °C / 48 h. Supernatants were used for determination of Co^{2+}, Mn^{2+} and Cr^{6+} concentrations by UV-VIS spectrophotometric methods (Fries and Getrost, 1977; Zeiner *et al*., 2010). All determinations were done in triplicate and analytical errors were in the range of 3.2 - 5.6%. Metal ion concentrations in the suspended biomass as well as in the microbial biofilm were determined with an inductively coupled plasma - mass spectrometer (ICP-MS). The instrument was equipped with a standard one piece extended torch with a quartz injector tube, a cyclone spray chamber and a GemCone nebulizer. The biofilm samples for ICP-MS were prepared in a closed microwave digestion system using a mixture of nitric acid (5 mL), hydrogen peroxide (1 mL) and doubly distilled water (1 mL). The samples were digested according to the following program (power [W]/time [min]): 250/3, 0/1, 250/4.5, 650/6, 400/5, ventilation 25.0 min and measured by ICP-MS.

Biofilm thickness measurements were done by a modified Venkataraman and Ramanujam (1998) method. The modifications were the use of graphite powder instead of chalk powder and microscope with a micrometric scale in place of the projector, respectively. The mass of biofilm was determined by collecting the biofilm sample from the inner bioreactor surface. The sample was suspended in demineralised water, centrifuged and washed twice with demineral-

ised water and then dried at 105 °C / 48 h.

Biofilm density ($c_{x,f}$) was calculated from the measured dry weight of the attached microbial biomass by using the following equation:

$$c_{x,f} = \frac{m_{x,f}}{S \cdot L_f} \qquad (\text{kg m}^{-3}) \tag{1}$$

where $m_{x,f}$ - dry weight of microbial biomass in the biofilm, S - inside bioreactor surface and L_f - biofilm thickness.

Suspended biomass ($q_{x,L}$) and biofilm ($q_{x,f}$) sorption capacity were calculated as follows:

$$q_{x,L} = \frac{m_M}{m_{x,L}} \quad \text{and} \quad q_{x,f} = \frac{m_M}{m_{x,f}} \quad (\text{mg g}^{-1}) \tag{2}$$

where m_M - mass of metal ion and $m_{x,L}$ - dry weight of suspended microbial biomass.

Volumetric removal rate of metal ions (Q_M) was estimated by Equation (3).

$$Q_M = \frac{c^0{}_{M,L} - c^{out}{}_{M,L}}{t_R} \qquad (\text{kg m}^{-3}\ \text{h}^{-1}) \tag{3}$$

where $c^0_{M,L}$ - metal ion concentration in the bioreactor inflow, $c^{out}_{M,L}$ - metal ion concentration in the bioreactor outflow and t_R - retention time.

For description of the HRTB performance, the integral bioprocess model was used that combines mixing, mass transfer and bioprocess kinetics. The structured cascade "spiral flow" model was used for the mixing description in the HRTB (Šantek *et al*., 1996). Mass balances of metal ions for the first ideally mixed compartment of the first cascade (Figure 1a) were:

$$V_L^{1,1}\frac{dc^{1,1}_{M,L}}{dt} = F_u c^0_{M,L} + F_{cr} c^{1,Ni}_{M,L} + F_p c^{2,1}_{M,L} - (F_u + F_p)c^{1,1}_{M,L} - F_{cr} c^{1,1}_{M,L} - V_L^{1,1} r^{1,1}_{M,L} \tag{4}$$

where $V_L^{1,1}$ - liquid volume in the first compartment (Ni=1) of first cascade (Nl=1), $c^{1,1}_{M,L}$ - metal ion concentration in the first compartment (Ni=1) of the first cascade (Nl=1) in the liquid phase, F_u - bioreactor inflow, F_{cr} - circulation flow, $c^{1,Ni}_{M,L}$ - metal ion concentration in the Ni-compartment of first cascade (Nl=1) in the liquid phase, F_p - back flow, $c^{2,1}_{M,L}$ - metal ion concentration in the first compartment (Ni=1) of the second cascade (Nl=2) in the liquid phase, $r^{1,1}_{M,L}$ - reaction rate in the first compartment (Ni=1) of first cascade (Nl=1) in the liquid phase and t - time.

First ideally mixed compartments of all cascades in the mixing model are assumed to be without a biofilm layer (Figure 1a). All other ideally mixed compartments inside the cascade are in direct contact with the biofilm layer (Figure 1b). Therefore, mass balances of metal ions are determined for the second (or other) ideally mixed compartments of the cascade (Figure 1a, b) as follows:

$$V_L^{1,2}\frac{dc^{1,2}_{M,L}}{dt} = F_{cr} c^{1,1}_{M,L} - F_{cr} c^{1,2}_{M,L} - S^{1,2} k_m (c^{1,2}_{M,L} - c^{1,2}_{M,f(Z=0)}) - V_L^{1,2} r^{1,2}_{M,L} \tag{5}$$

where $V_L^{1,2}$ - liquid volume in the second compartment (Ni=2) of the first cascade (Nl=1), $c^{1,2}_{M,L}$ - metal ion concentrations in the second compartment (Ni=2) of the first cascade (Nl=1) in the liquid phase, $S^{1,2}$ - the surface for mass transfer in the second compartment (Ni=2) of the first cascade (Nl=1), k_m - mass transfer coefficient in the stagnant liquid layer, $c^{1,2}_{M,f(Z=0)}$ - metal ion concentration in the second compartment (Ni=2) of the first cascade (Nl=1) on the biofilm surface, $r^{1,2}_{M,L}$ - reaction rate in the second compartment (Ni=2) of the first cascade (Nl=1) in the liquid phase and t - time.

Metal ion concentrations in the bulk liquid phase ($c_{M,L}$) of the compartment were assumed to be constant for each ideal mixed compartment (Figure 1a). In this model, it was also assumed that the biofilm is submerged in the stagnant liquid layer (L_g, Figure 1b). The mass transfer processes in the liquid phase and inside the microbial biofilm on the inner surfaces of the HRTB were defined as described earlier (Rezić *et al*., 2011b).

In this model, the substrate consumption rate in the biofilm ($r^{1,2}_{M,f}$) is assumed to be equal to the mass transfer of all dissolved metal ions in the biofilm:

$$D_{ef,M}\frac{\partial^2 c^{1,2}_{M,f}}{\partial z^2} = r^{1,2}_{M,f} \tag{6}$$

where $D_{ef,M}$ - effective diffusion coefficient of the metal ion in the biofilm, $c^{1,2}_{M,f}$ - metal ion concentrations in the second compartment (Ni=2) of the first cascade (Nl=1) in the biofilm and z - biofilm depth coordinate.

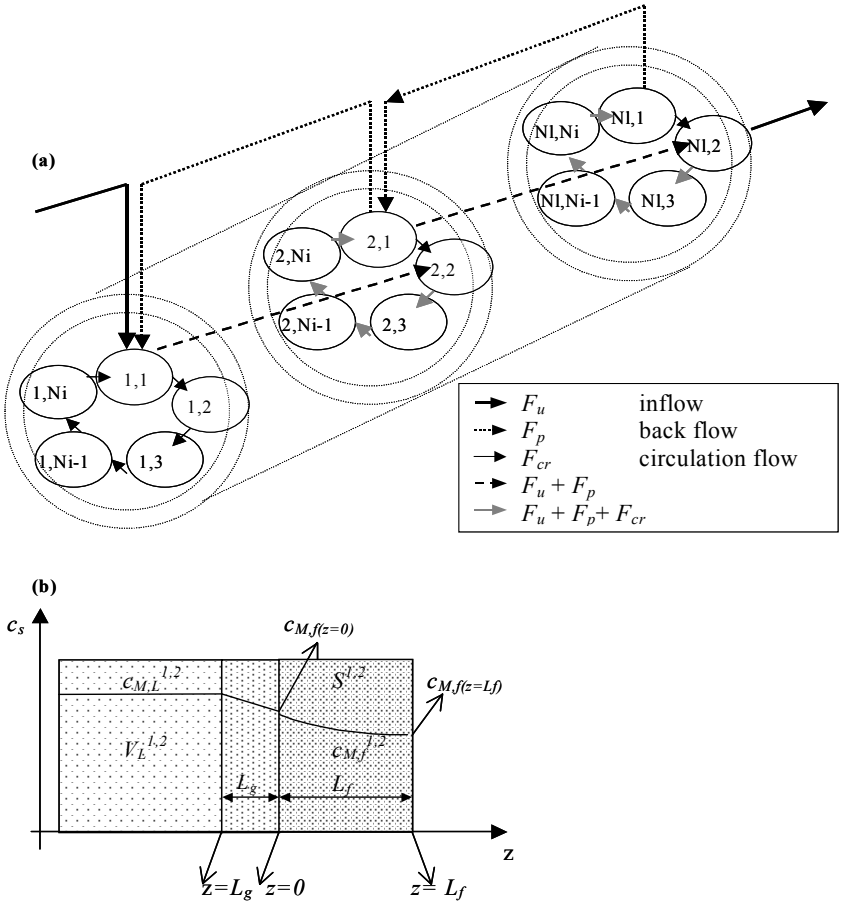

Figure 1: "Spiral flow" mixing model of the HRTB (a) and metal ion concentration profile in the second (*Ni*=2; b) ideally mixed compartment of the first cascade (*Nl*=1) in the mixing model.

The outer boundary conditions (at $z = 0$) at the liquid - biofilm interface are given as:

$$S^{1,2}k_m(c_{M,L}^{1,2} - c_{M,f(z=0)}^{1,2}) = S^{1,2}D_{ef,M}\frac{dc_{M,f}^{1,2}(z)}{dz}\Big|_{z=0} \quad (7)$$

The inner boundary conditions (at $z = L_f^{1,2}$) at the biofilm-bioreactor wall (or discs) interface are given as:

$$0 = \frac{dc_{M,f}^{1,2}(z)}{dz}\Big|_{z=L_f^{1,2}} \quad (8)$$

Metal ion concentrations in the biofilm ($c_{M,f}$) are presented as functions of biofilm depth z (Figure 1b) and are given as a second-order polynomial equation:

$$c_{M,f}(z) = a_0 + a_1 z + a_2 z^2 \quad (9)$$

where a_0, a_1 and a_2 are the second-order polynomial correlation coefficients.

For the compartments with biofilm layer in the state of dynamic equilibrium at time (t), the model was derived from mass balance equations (Equations (5), (6)- (8)) and the second-order polynomial correlation for description of the metal ion concentrations (Equation (9)) across the biofilm layer. For more details see Rezić *et al*. (2011b).

Mass balance equations of the integral bioprocess model were coupled to the reaction rates in the liquid phase ($r_{M,L}$) and in the biofilm layer ($r_{M,f}$), respectively. Instead kinetic terms for heavy metal removal were estimated by Freundlich adsorption isotherm (Equations (10) - (11)):

$$q_{x,L} = K_{F,L}(c_{M,L})^{1/h_L} \quad (10)$$

where $q_{x,L}$ - suspended biomass adsorption capacity, $K_{F,L}$ - Freundlich isotherm constant for suspended microbial biomass, $c_{M,L}$ - metal ion concentration in the liquid phase and h_L - Freundlich isotherm constant for suspended microbial biomass.

$$q_{x,f} = K_{F,f}(c_{M,f})^{1/h_f} \tag{11}$$

where $q_{x,f}$ - biofilm adsorption capacity, $K_{F,f}$ - Freundlich isotherm constant for microbial biofilm, $c_{M,f}$ - metal ion concentration in the biofilm and h_f - Freundlich isotherm constant for microbial biofilm.

The kinetic model of the biofilm layer was derived from the Freundlich isotherm (Equation (11)) and the second-order polynomial correlation and it has following form:

$$q_{x,f} = K_{F,f}\left[a_0 + a_1 z_i + a_2 (z_i)^2\right]^{1/h_f} \tag{12}$$

z_i is collocation point across the biofilm layer parallel to the substrate surface ($z_i = L_f/p$ where p=2).

The kinetic model assumes that the reaction rate is a function of biomass concentration in the liquid phase ($c_{x,L}$) and in the biofilm layer ($c_{x,f}$):

$$r_{M,L} = \frac{c_{x,L} q_{x,L}}{t_R} \tag{13}$$

where $r_{M,L}$ - reaction rate in the liquid phase and t_R - retention time.

$$r_{M,f} = \frac{c_{x,f} q_{x,f}}{t_R} \tag{14}$$

where $r_{M,f}$ - reaction rate in the biofilm and t_R - retention time.

A system of differential equations was obtained by establishing mass balances for each ideally mixed compartment of the physical model (Figure 1a) and consequently the concentration of each metal ion along the HRTB can be determined.

Established model equations were solved by using the software "Wolfram Mathematica". The orthogonal collocation method was used for description of the inner biofilm metal ion concentration profiles (Arora *et al*., 2006). As orthogonal polynomial, the second-order polynomial equation was used. After preliminary determinations, optimization was performed by calculating the global minimum of variance (E_n) between experimental and simulated variables (using the software "Wolfram Mathematica") by the following equation:

$$E_n = \frac{1}{n_u} \sum_{i=1}^{i=n_u} \frac{(c^i_{n,\exp} - c^i_{n,sim})^2}{c^i_{n,\exp}} \tag{15}$$

where n_u - number of observations, $c^i_{n,\exp}$ - experimental variables and $c^i_{n,sim}$ - simulated variables.

RESULTS AND DISCUSSION

In this research, the dynamics of heavy metal (Co^{2+}, Mn^{2+} and Cr^{6+}) removal with mixed microbial culture in the HRTB was studied with different combinations of bioreactor process parameters (n and F). Changes of bioreactor process parameters (n and F) during this investigation were in accordance with our previous research (Rezić *et al*., 2011a). In order to obtained microbial culture for this research, an isolation procedure was performed by using soil samples extremely polluted with heavy metals (see Materials and methods). The assumption was that microbial cultures present in these soil samples are capable to grow on the medium with relatively high concentrations of heavy metals (Kavamura and Esposito, 2010). Due to the relatively long research time in the HRTB, it was necessary additionally to protect the bioprocess performance by using a relatively low pH of the medium (4.2-4.6). During research in the HRTB, the morphological characteristics of the microbial species were also monitored and it was observed that cocci and rods were the dominant microbial species in the suspended biomass, as well as filamentous bacteria in the microbial biofilm, respectively.

The bioprocess performance in the HRTB was monitored by the metal ion removal efficiency. The average volumetric metal ion removal rate (Q_M) as a function of the inflow rate (F) and bioreactor rotation speed (n) are presented in Table 1. The volumetric metal ion removal rate (Q_M) varied from 3.363 to 8.324 mg L^{-1} h^{-1} at inflow rates up to 1.0 L h^{-1}. The increase of the inflow rate to 2.0 L h^{-1} was related to the increase of the volumetric removal rate from 14.530 to 16.617 mg L^{-1} h^{-1} as a consequence of the higher metal ion volumetric load and the decrease of mass transfer resistance in these conditions (Kogej *et al*., 2010).

In this investigation, the metal ion removal efficiency was at approximately the same level for all metal ions examined [cobalt ion (89.0-95.7%), manganese (87.0-93.6%) and chromium (99.7-100%)] (Table 1). On the basis of these results it is clear that the microbial biomass (suspended biomass and

biofilm) in the HRTB has relatively high affinity for all three metal ions.

The increase of the inflow rate to 2.0 L h^{-1} was related to the approximately two-fold increase of the volumetric removal rate (6.67-16.6 mg L^{-1} h^{-1}) as a consequence of the higher metal ion volumetric load. In this investigation, the highest volumetric productivity of metal removal was observed for chromium (4.17-16.60 mg L^{-1} h^{-1}) compared to cobalt (3.98-13.7 mg L^{-1} h^{-1}) and manganese (3.38-14.99 mg L^{-1} h^{-1}; Table 1). On the basis of these results, it is shown that the biofilm in the HRTB has the highest affinity for chromium ion removal. This phenomenon can be explained by transport limitations due to the ion molar volume, which is smaller for chromium than for cobalt and manganese. Furthermore, the covalent index of chromium and its diffusion rate through the microbial biofilm are higher compared to the other two metal ions examined (Kogej and Pavko, 2001).

Table 1: Average volumetric removal rate (Q_M) and removal efficiency (E_M) of metal ions at different combinations of bioreactor process parameters (n and F).

F (L h^{-1})	n (min^{-1})	Q_M (mg L^{-1} h^{-1})			E_M (%)		
		Co^{2+}	Cr^{6+}	Mn^{2+}	Co^{2+}	Cr^{6+}	Mn^{2+}
0.5	5	3.598	3.598	3.873	31.0	99.96	93.1
	15	3.363	3.363	3.859	60.3	99.87	92.7
	30	3.454	3.454	3.893	68.5	99.90	93.5
1.0	5	7.523	7.523	7.811	41.1	99.82	93.7
	15	7.478	7.478	7.797	48.8	99.84	93.5
	30	7.781	7.781	7.804	58.8	99.77	93.6
2.0	5	15.569	15.569	14.663	40.3	99.68	87.6
	15	15.065	16.609	14.530	42.1	99.68	87.0
	30	15.128	16.617	14.663	40.7	99.73	88.0

Similar investigation of nickel and chromium biosorption was done by Barroso *et al*. (2006) in the UASB reactor with sanitary sewage sludge. Removal efficiency was in the range of our results. Samarth *et al*. (2012) investigated the biosorption of a multi-metal aqueous solution on the living biomass of *Bacillus licheniformis* which was already used in the sewage treatment process. Aerobic batch biosorption experiments were carried out for removal of Cr (VI), Fe (III), Cu (II) ions from aqueous multi-metal solutions. Removal efficiencies for iron, chromium and copper ion were 95, 52 and 32% after 48 hours of bioprocess, respectively. Gomathi *et al*. (2012) investigated the biosorption of chromium by *Aplanochytrium* sp. Maximum removal of chromium (69.4%) was observed in the batch system with the following bioprocess conditions: pH range of 6.8 - 8.2, T = 27 °C and adsorbent dosage (dried biomass of *Aplanochytrium* sp.) of 0.51 g L^{-1}. In the study of Zhou *et al*. (2007) the removal of Cr (VI) from aqueous solution was studied in a batch system by the sorption on the dead cells of *Bacillus licheniformis* isolated from metal-polluted soils. The maximum biosorption capacity of Cr (VI) ion (60.5 mg/g) was observed under the following conditions: pH = 2.5, Cr (IV) concentration = 300 mg L^{-1}, biomass concentration = 1 g L^{-1}, T = 50 °C and contact time = 2 h. In the study of Quintelas *et al*. (2009b) the following removal efficiencies were observed: iron (100%), cadmium (70%), nickel (40 - 74%) and chromium (20 - 100%) in the batch system by sorption on the biofilm of *Escherichia coli* supported on kaolin. *Arthrobacter viscosus* biofilm supported on granular activated carbon in a batch system was also examined. Six isotherms were tested and the best description of the experimental data was observed for the Freundlich isotherm. In this research, removal efficiency was reduced with an increase of the initial chromium concentration (95.20% for 5 mg L^{-1} and 38.28% for 1000 mg L^{-1}). The batch adsorption studies were used to develop a pilot scale bioreactor which has the capacity for complete chromium removal from aqueous solutions (average chromium removal efficiency was 99.9% in the first 30 days of the bioprocess; Quintelas *et al*. 2009a). Three different species of nonliving red algal biomass *Laurancia obtusa*, *Geldiella acerosa* and *Hypnea sp*. were used in a fixed-bed column bioreactor for removal of toxic heavy metal ions [Cu (II), Zn (II), Mn (II) and Ni(II)] from industrial effluent. The highest efficiency of metal ion removal was detected in the bioreactor with *L. obtusa* (94%), followed by *G. acerosa* (85%) and *Hypnea sp.* (71%; Wael and Hawazin 2013).

Suspended biomass concentrations obtained for different combinations of bioreactor process parameters (n and F) at the outlet of HRTB are presented in Table 2. The suspended biomass concentrations ($c_{x.L}$) were in the range of 0.72-1.28 g L^{-1} (previous research 1.66-1.95 g L^{-1}) at an inflow rate of 0.5 L h^{-1}. Furthermore, the increase of the inflow rate to 1.0 and 2.0 L h^{-1} was related to the increase of suspended biomass concentrations to 1.10-6.32 g L^{-1} (previous research 2.05-3.48 g L^{-1}) as a consequence of biofilm erosion (continuous release of smaller biofilm parts). The highest detected suspended biomass concentration in the HRTB was 6.32 g L^{-1} as a consequence of intensive biofilm detachment (release of larger biofilm parts) due to experimental conditions (n = 30 min^{-1} and F = 2.0 L h^{-1}) that considerably stimulated this process. In this case, a considerable increase of metal ion concentrations in the liquid phase was observed as a consequence of

biomass washout from the HRTB. On the basis of the obtained results it is clear that the bioreactor rotation speed did not have a pronounced effect on the suspended biomass concentration. These observations are in agreement with our previous research (Ivančić *et al*., 2004; Rezić *et al*., 2011a). However, some discrepancies from the above mentioned effect were observed at F= 0.5 L h^{-1} and n = 30 min^{-1} as a consequence of analytical errors.

Table 2: The suspended biomass concentrations ($c_{x,L}$) and sorption capacity ($q_{x,L}$) at different combinations of bioreactor process parameters (*n* and *F*) during heavy metals removal process in the HRTB.

F (L h^{-1})	*n* (min^{-1})	L_{HRCB} (%)	$c_{x,L}$ (g L^{-1})	$q_{x,L}$ (mg g^{-1})		
				Co^{2+}	Cr^{6+}	Mn^{2+}
0.5	5	0	0.85	31.004	3.746	29.451
0.5	5	50	1.14	33.981	10.849	37.737
0.5	5	100	1.28	6.834	1.691	7.313
0.5	15	0	0.72	4.518	2.632	4.494
0.5	15	25	0.78	8.251	4.013	8.470
0.5	15	50	0.83	9.256	5.304	10.630
0.5	15	75	1.14	7.907	1.545	8.576
0.5	15	100	1.23	7.530	1.369	8.067
0.5	30	0	0.96	19.389	4.365	19.729
0.5	30	50	1.05	21.230	7.744	23.992
0.5	30	100	1.09	2.044	0.677	2.092
1.0	5	0	1.10	1.654	0.200	1.708
1.0	5	50	1.62	13.917	1.832	14.540
1.0	5	100	2.05	7.742	1.582	9.096
1.0	15	0	1.38	29.249	2.489	33.762
1.0	15	25	1.42	17.998	3.711	22.532
1.0	15	50	1.86	14.732	1.948	17.850
1.0	15	75	2.21	18.239	2.408	22.570
1.0	15	100	2.33	24.280	2.699	29.509
1.0	30	0	1.78	27.640	2.269	28.994
1.0	30	50	2.64	34.788	4.189	40.848
1.0	30	100	2.89	32.935	3.255	39.958
2.0	5	0	1.91	36.926	3.050	40.558
2.0	5	50	2.96	16.743	5.309	21.591
2.0	5	100	3.13	21.788	2.201	28.031
2.0	15	0	2.02	49.712	7.027	42.970
2.0	15	25	2.57	17.163	6.169	23.099
2.0	15	50	3.03	8.866	5.976	13.603
2.0	15	75	3.25	5.568	5.508	8.984
2.0	15	100	3.59	6.289	5.516	9.902
2.0	30	0	3.82	57.160	4.295	59.010
2.0	30	50	5.20	28.427	1.653	34.266
2.0	30	100	6.32	27.673	2.293	35.050

The suspended biomass sorption capacity ($q_{x,L}$) during this research is also presented in Table 2. The highest bioreactor rotation speed (30 min^{-1}) decreased the thickness of the stagnant liquid-layer at the biomass surface and facilitated conditions for metal ion sorption. Higher biomass sorption capacity was observed at inflow rates of 1.0 and 2.0 L h^{-1} as a consequence of biofilm erosion. The content of the biofilm in the suspended biomass has a significant impact on the suspended biomass sorption capacity. Biofilm structure and the extracellular polymeric substance (EPS) content increase the possibility of ion sorption (Guibaud *et al*., 2009; Ozturk *et al*., 2009; Ni *et al*., 2010). EPS is polyanionic in nature, with enhanced capability for cation ion bonding. Moreover, enzymatic activities in EPS also assist in the detoxification of heavy metals by transformation and subsequent precipitation in the EPS (Pal and Paul, 2008).

The sorption capacity of suspended biomass ($q_{x,L}$) was higher for cobalt and manganese ions than for chromium ion. This effect can be explained by the fact that cobalt and manganese ions have a slightly higher covalent index (Chen and Wang, 2007) as well as the pH gradient along the HRTB (Baes and Mesmer, 1976). In this investigation, at an inflow rate of 0.5 L h^{-1}, the pH gradient along the HRTB was in the range of 4.43 - 4.72. Increase of inflow rate to 1 and 2 L h^{-1} was related to the increase of the pH gradient along the HRTB in the range of 4.19-5.82 (Table 3).

Table 3: pH variations along HRTB at different combinations of bioreactor process parameters (*n* and *F*) during the heavy metal removal process.

F (L h^{-1})	*n* (min^{-1})	pH (0% L_{HRCB})	pH (50% L_{HRCB})	pH (100% L_{HRCB})
0.5	5	4.56	4.68	4.71
0.5	15	4.43	4.52	4.68
0.5	30	4.52	4.72	4.72
1.0	5	4.51	4.92	5.29
1.0	15	4.39	4.86	5.37
1.0	30	4.29	4.98	5.21
2.0	5	4.31	5.12	5.72
2.0	15	4.19	5.25	5.82
2.0	30	4.25	5.43	5.69

On the basis of these results it is clear that the inflow rate has a more pronounced effect on the pH gradient than bioreactor rotation. Metal ion sorption on the microbial biomass is pH dependent, as the pH affects the availability of metal ions in solution (speciation), as well as the metal binding sites on the cell surface. As the pH is increased along the HRTB due to the medium consumption, the overall surface charge of microbial cell will become negative, resulting in more efficient cobalt and manganese sorption. In aqueous solution Cr^{6+} ion appears as an oxyanion (CrO_4^{2-} or $Cr_2O_7^{2-}$) and therefore it can not bind effectively to a negatively charged microbial

cell surface. The Cr^{6+} ion sorption process in the HRTB was mostly carried out only on the first 50% of bioreactor length where the pH was below 5 due to the fact that the overall microbial cell surface charge becomes more positive, promoting sorption of anionic Cr^{6+} ion species (Baes and Mesmer, 1976; Zhou *et al*., 2007). Furthermore, hydrodynamic conditions in the HRTB also affected all previously mentioned factors (Gavrilescu, 2004). Therefore, on the basis of these results it is clear that biological and hydrodynamic conditions in the HRTB have a significant effect on the suspended biomass concentration and its sorption capacity (Table 2).

Biofilm sorption capacity ($q_{x,f}$) and biofilm volumetric density ($c_{x,f}$) at the inlet and the outlet of the HRTB were determined at $n = 30$ min^{-1} and $F = 2.0$ L h^{-1} (Table 4). The HRTB is characterized by a concentration gradient along the bioreactor, so consequently a higher volumetric biofilm density was observed at the inlet of the HRTB (59.7±5.2 g L^{-1}) than at the outlet of the HRTB (39.3±4.4 g L^{-1}). This effect can be explained by the fact that higher substrate concentrations for microorganism growth are present at the inlet than at the outlet of the HRTB. The structure and the content of the biofilm have a significant effect on the biofilm density and sorption capacity. Microbial sorption capacity is closely related to the content and molecular size of EPS in the biofilm. Due to these facts, the outer biofilm layers have higher porosity and provide relatively easy metal ion access to deeper biofilm layers reached with EPS. Therefore, high volumetric density biofilms have higher sorption capacity than low density biofilms characterized by the low content of EPS (Cloirec *et al*., 2003).

Table 4: Biofilm sorption capacity ($q_{x,f}$) and volumetric density ($c_{x,f}$) at the inlet and outlet of the HRTB for $F = 2.0$ L h^{-1} and $n = 30$ min^{-1}.

$q_{x,f}$ (mg g^{-1})	L_{HRCB} (%)	
	0	100
Co^{2+}	37.123	20.652
Cr^{6+}	14.512	5.649
Mn^{2+}	49.217	49.233
$\Sigma q_{x,f}$ (mg g^{-1})	100.852	49.233
$c_{x,f}$ (g L^{-1})	59.7±5.2	39.3±4.4

At $F = 2.0$ L h^{-1} and $n = 30$ min^{-1} the lowest biofilm sorption capacity was observed for chromium ion compared to the other metal ions examined (Table 4). As in the case of suspended biomass, this effect can be explained by the metal covalent index, the medium and biofilm characteristics and the hydrodynamic conditions in the HRTB (Kogej and Pavko, 2001; Cloirec *et al*., 2003; Chen and Wang, 2007; Chen and Wang, 2008). The main impact on the biofilm sorption capacity is from the covalent index of the metal ions and the biofilm volumetric density. Cobalt and manganese ions have slightly higher covalent index than chromium ion and therefore metal ions with higher covalent index have higher potential to form bonds with biological ligands in the biofilm (Kogej and Pavko, 2001; Chen and Wang, 2007). The structure and the content of the biofilm are responsible for biofilm volumetric density, which affects biofilm sorption capacity (Hall-Stoodley and Stoodley, 2002; Liu and Tay, 2002; Cloirec *et al*., 2003). Hydrodynamic conditions are closely related to the shear stress and abrasion process in the HRTB. Therefore, the outer biofilm layers are more sensitive to these two effects than the inner biofilm layers. Outer biofilm layers can be released even at relatively small shear stress (Donlan and Costerton, 2002; Liu and Tay, 2002).

Biofilm thickness along the HRTB obtained for different combinations of medium inflow rates (F = 0.5-2.0 L h^{-1}) and bioreactor rotation speed (n = 5-30 min^{-1}) are presented in Table 5. The biofilm thickness was in the range of 0.23-1.43 mm, which is thinner compared to the literature data for mixed culture biofilm, but thicker compared to mono-microbial culture biofilms (Rezić *et al*., 2007). The bioreactor rotation speed did not have a significant effect on the biofilm thickness (Table 5). The effect of medium inflow rate (F) on the biofilm thickness was more obvious at 2.0 L h^{-1} where biofilm erosion is intensified, combined with the decrease of biofilm thickness. It is well known that lower medium inflow rates favor the formation of thicker biofilms with lower areal biofilm density. The increase of inflow rate produces thinner biofilm with higher areal density (Gavril and Macoveanu, 2000; Donlan and Costerton, 2002; Liu and Tay, 2002).

Table 5: Biofilm thickness changes (L_f) along the HRTB at different combinations of bioreactor process parameters (n and F) during the heavy metal removal process.

F (L h^{-1})	n (min^{-1})	L_f (mm)		
		(0% L_{HRCB})	(50% L_{HRCB})	(100% L_{HRCB})
0.5	5	0.75	1.08	0.89
	15	0.89	0.73	0.81
	30	0.93	1.29	0.85
1.0	5	0.85	1.34	0.95
	15	0.92	1.43	0.84
	30	0.86	1.21	0.91
2.0	5	0.23	0.37	0.28
	15	0.35	0.28	0.25
	30	0.38	0.37	0.35

The biofilm thickness in the HRTB (L_f) was mostly stabile at inflow rates of 0.5 and 1 L h^{-1}, and only smaller biofilm parts were observed in the liquid phase as a consequence of the biofilm erosion process. The highest biofilm thickness was observed in the first 50% of bioreactor length as a consequence of substrate ability in this part of HRTB. Increase of the inflow rate to 2 L h^{-1} was related to the intensification of the biofilm erosion process as a result of higher shear stress and metal load in these conditions that were related to the decrease of biofilm growth and thickness. On the basis of these results, it is clear that the increase of the inflow rate and metal ion concentration produces thinner biofilms with higher density.

One of the goals of this research was to evaluate the integral bioprocess model for heavy metals removal in the HRTB. The established integral bioprocess model combines the mixing model and a metal ion diffusion-adsorption model. A diffusive biofilm concept was introduced in order to detect changes of metal ion concentration inside the microbial biofilm attached to the inner surfaces of the HRTB (Rezić *et al*., 2011b).

In this research, the integral bioprocess model was used for description of the manganese (Mn^{2+}), cobalt (Co^{2+}) and chromium (Cr^{6+}) removal process in the HRTB. Due to the fact that experiments were performed by the same combinations of bioreactor process parameters (n and F) the same optimal values of mixing model parameters (Nl, Ni, F_{cr} and F_p) were used in the simulation of bioprocess behavior in the HRTB (Table 6). For more details related to the mixing model parameters and their effect on the integral bioprocess model prediction efficiency see Rezić *et al*., (2011b).

Table 6: Optimal values of adjustable mixing model parameters for different combinations of bioreactor process parameters (n and F) utilized in the integral bioprocess model (Rezić *et al*., 2011b).

F (L h^{-1})	n (min^{-1})	Nl	Ni	F_{cr} (m^3 h^{-1})	F_p (m^3 h^{-1})
0.5	5	5	3	$6.23\cdot10^{-4}$	$9.22\cdot10^{-3}$
	15	5	3	$7.04\cdot10^{-4}$	$5.33\cdot10^{-3}$
	30	4	3	$4.29\cdot10^{-3}$	$1.02\cdot10^{-3}$
1.0	5	5	3	$5.64\cdot10^{-4}$	$9.34\cdot10^{-3}$
	15	5	3	$8.07\cdot10^{-4}$	$5.23\cdot10^{-3}$
	30	4	3	$5.01\cdot10^{-3}$	$9.61\cdot10^{-4}$
2.0	5	8	6	$8.95\cdot10^{-4}$	$8.89\cdot10^{-3}$
	15	8	6	$2.41\cdot10^{-3}$	$4.78\cdot10^{-3}$
	30	7	5	$5.83\cdot10^{-3}$	$9.09\cdot10^{-4}$

The effective diffusion coefficients of metal ions in the biofilm ($D_{ef,M}$) were calculated by previously established procedure and presented in Table 7 (Rezić *et al*., 2011b). Obtained effective diffusion coefficients ($D_{ef,M}$) are in the range of literature data (Camur and Yazicigil, 2005).

Table 7: Values of effective diffusion coefficients for different metal ions in the microbial biofilm on the inner surface of HRTB.

Parameter	Value (m^2 s^{-1})
$D_{ef.Co}$	$9.2\cdot10^{-10}$
$D_{ef.Cr}$	$11.2\cdot10^{-10}$
$D_{ef.Mn}$	$8.9\cdot10^{-10}$

Mass transfer rate coefficients of metal ions in the HRTB (k_m) were determined by a previously established procedure and presented in Table 8 (Rezić *et al*., 2011b). The increase of bioreactor rotation speed (n) reduces the thickness of the stagnant liquid layer near the microbial biofilm in the HRTB and enlarges the Reynolds rotation number, which contributes to more efficient transfer of metal ions through the stagnant liquid layer. Therefore, the increase of bioreactor rotation speed (n) was related to the increase of the mass transfer rate coefficient (k_m).

Table 8: Mass transfer rate coefficients calculated for different bioreactor rotation speeds.

n (min^{-1})	k_m (m h^{-1})
5	0.356
10	0.411
15	0.498
20	0.535
30	0.628

For description of metal ion adsorption in the suspended biomass, the Freundlich isotherm was selected because of its better description of bioprocess performance in the HRTB (Rezić *et al*., 2011b). In this study, the adjustable parameters of Freundlich isotherm for all examined metal ions are estimated by the procedure described earlier (Rezić *et al*., 2011b) and presented in Table 9. Results obtained are in the range of our previous research (Rezić *et al*., 2011b).

The Freundlich isotherm was also used for description of the metal ions adsorption process inside the microbial biofilm on the inner surface of the HRTB. In the integral bioprocess model it is assumed that inside the microbial biofilm attached on the inner surface of the HRTB concentration profiles have concave forms, in agreement with literature data (Wik *et al*., 2005; Ntwampe *et al*., 2008). Values of adjustable parameters of Freundlich isotherm for the adsorption process inside the microbial biofilm in the HRTB are also presented in Table 9.

Table 9: Parameters of the Freundlich adsorption model for suspended biomass and microbial biofilm on the inner surface of the HRTB for different metal ions in the multi-metals solution.

Parameter	Suspended biomass	Microbial biofilm
h_{Co}	1.202	1.352
$K_{F.Co}$	0.949	1.245
h_{Cr}	1.578	1.742
$K_{F.Cr}$	0.793	1.128
h_{Mn}	1.958	2.034
$K_{F.Mn}$	1.343	1.772

Optimization of biofilm adsorption parameters was carried out by using the minimum of variance between experimental and simulated values of metal ion liquid concentrations in the HRTB as a criterion. Relations between the alteration of adsorption model parameters ($K_{F,f}$ and h_f) and the variance (E_n) were estimated by the second-order regression curve, as shown in Figure 2 (as the example Mn^{2+} ion was selected). For Mn^{2+} ion the minimum of the fitted curve was chosen as the optimum for $K_{F,f}$ and h_f (Figure 2) values, respectively.

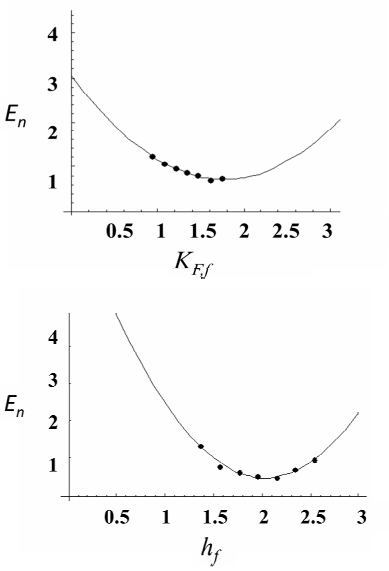

Figure 2: Relation between the alteration of the Freundlich model parameters ($K_{F,f}$ and h_f) and the variance (E_n) for Mn^{2+} ion

A similar procedure was also used for all other metal ions and the results are presented in Table 9. Because of the bioprocess complexity, which is affected by different environmental conditions (e.g. medium composition and pH gradient along the bioreactor, composition and physiological state of suspended biomass and biofilm along the bioreactor), the previously described procedure was established in order to take into account all these effects on the bioprocess performance efficiency in the HRTB. For more details related to the adsorption model parameters and their effect on the integral bioprocess model prediction efficiency see Rezić *et al*., (2011b).

Changes of metal ion concentrations in the HRTB were simulated for the entire operation period by using the established integral bioprocess model that combines mixing, mass transfer and bioprocess kinetics. The global minimum of variance between experimental and simulated values of metal ions concentration in the HRTB was the criterion for the evaluation of the integral bioprocess model prediction efficiency. Obtained variance values for different combinations of the bioreactor process parameters are presented in Table 10. As can be seen in Table 10, variance values for different metal ions were in the following range: E_{Co} = 0.12 - 1.41·10^{-3}; E_{Cr} = 0.34 - 1.11·10^{-3}; E_{Mn} = 0.71 - 3.21·10^{-3}. Obtained results were in the range of our previous research (Rezić *et al*., 2011b). However, these results also pointed out that changes of both bioreactor process parameters (n and F) did not have a significant impact on the integral bioprocess model prediction efficiency.

Table 10: Variance between experimental and simulated metal ion concentrations for different combinations of bioreactor process parameters (n and F) using the integral bioprocess model.

Co^{2+}	$E_{Co} \cdot 10^{-3}$		
n (min^{-1})	**5**	**15**	**30**
F (L h^{-1})			
0.5	0.12	0.87	0.16
1.0	0.28	1.30	0.32
2.0	0.32	1.41	0.72
Cr^{6+}	$E_{Cr} \cdot 10^{-3}$		
n (min^{-1})	**5**	**15**	**30**
F (L h^{-1})			
0.5	0.34	0.44	0.79
1.0	0.56	0.77	0.66
2.0	1.89	1.11	0.87
Mn^{2+}	$E_{Mn} \cdot 10^{-3}$		
n (min^{-1})	**5**	**15**	**30**
F (L h^{-1})			
0.5	0.71	1.05	1.44
1.0	0.75	3.21	0.97
2.0	0.80	1.98	0.80

Examples of metal ion profiles along the bioreactor length in the experiment with different medium inflow rates (F = 0.5 - 2.0 L h^{-1}) and constant bioreactor rotation speed (n = 15 min^{-1}) are presented in Figure 3 (Co^{2+} concentration Figure 3a; Cr^{6+} concentration Figure 3b; Mn^{2+} concentration Figure 3c). Points in these figures represented experimental

values and simulated curves the bioprocess model values, respectively. The metal ion concentrations in the inflow of the HRTB were 0.125 g L^{-1}. At the first measuring point (0% L_{HRCB}) metal ion concentrations were considerably lower due to the dilution effect and different metal ion sorption capacities. HRTB is characterized by plug-flow conditions and therefore a gradient of metal ion concentration along the bioreactor was observed (Figure 3; Šantek *et al*., 2000).

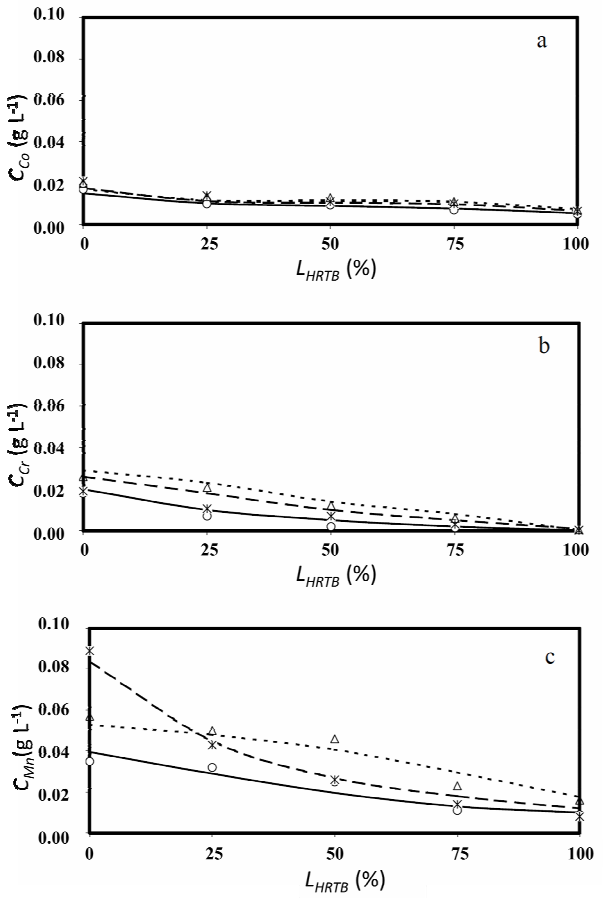

Figure 3: Simulations of Co^{2+} (a), Cr^{6+} (b) and Mn^{2+} (c) ion concentrations along the HRTB at different medium inflow rates F = 0.5 L h^{-1} (○, full line), F = 1.0 L h^{-1} (*, dashed line), F = 2.0 L h^{-1} (Δ, dotted line) and constant bioreactor rotation speed (n = 15 min^{-1}) using in the integral bioprocess model.

The highest metal ion concentrations were detected in the first 25% of the bioreactor length and they could considerably reduce biomass activity and consequently the metal ion removal efficiency. In this experiment, the impact of medium inflow rate on the Co^{2+} and Cr^{6+} concentration profiles was not statistically significant. However, this impact was more pronounced at other two bioreactor rotation speeds (5 and 30 min^{-1}). As mentioned earlier, the biofilm sorption is a complex process that is affected by hydrodynamic conditions, the morphological and physiological characteristics of the biofilm (Aksu *et al*., 1992; van Hullebusch *et al*., 2003) and the covalent index of the metal ions (Kogej and Pavko, 2001; Kogej *et al*., 2010; Chen and Wang, 2007). As can be seen in Figure 3, relatively good agreement between experimental and simulated values of the metal ion concentration along HRTB was observed. Similar agreement was also found at all other bioreactor rotation speeds (data not shown). On the basis of these results, it is clear that the established integral bioprocess model is capable to describe the heavy metal removal process in the HRTB.

CONCLUSIONS

Wastewater polluted with heavy metals is a serious ecological problem. In this research, a multi heavy metal removal process with mixed microbial culture was studied in the HRTB by different combinations of process parameters. Mixed microbial culture has the ability to remove heavy metals from wastewater polluted with multi-metal solution (total concentrations up to 375 mg L^{-1}). For the description of heavy metal removal in the HRTB, the integral bioprocess model was used, which combines hydrodynamics (mixing), mass transfer and kinetics. Mixing in the HRTB was described by the structured cascade model and metal ion removal by a combined diffusion-adsorption model, respectively. This bioprocess model was tested in new experimental conditions and the average variances between experimental and simulated metal ion concentrations were in the range of 0.12 - 3.21·10^{-3}. Obtained results clearly show that the established integral bioprocess model can describe the heavy metal removal process in the HRTB. On the basis of these results it is clear that the integral bioprocess model has great potential to be used in a scale-up procedure based on the geometrical, hydrodynamic and physiological similarity of the bioprocess on the small and large scale.

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NOMENCLATURE

a_0, a_1 and a_2	the second order polynomial correlation coefficient	
$c_{M,f}^{1,2}$	metal ion concentrations in the second compartment (*Ni*=2) of the first cascade (*Nl*=1) in the biofilm	kg m^{-3}
$c_{M,L}^{1,1}$	metal ion concentrations in the first compartment (*Ni*=1) of the first cascade (*Nl*=1) in the liquid phase	kg m^{-3}
$c_{M,L}^{1,Ni}$	metal ion concentrations in the *Ni*-compartment of the first cascade (*Nl*=1) in the liquid phase	kg m^{-3}
$c_{M,L}^{1,2}$	metal ion concentration in the second compartment (*Ni*=2) of the first cascade (*Nl*=1) in the liquid phase	kg m^{-3}
$c_{M,L}^{2,1}$	metal ion concentration in the first compartment (*Ni*=1) of the second cascade (*Nl*=2) in the liquid phase	kg m^{-3}
$c_{M,f(Z=0)}^{1,2}$	metal ion concentration in the second compartment (*Ni*=2) of the first cascade (*Nl*=1) on the biofilm surface	kg m^{-3}
$c_{M,L}^{0}$	metal ion concentration in the bioreactor inflow	kg m^{-3}
$c_{M,L}^{out}$	metal ion concentration in the bioreactor outflow	kg m^{-3}
$c_{M,f}$	metal ion concentration in the biofilm	kg m^{-3}
$c_{M,L}$	metal ion concentration in the liquid phase	kg m^{-3}
$c_{n,\exp}^{i}$	experimental variables	kg m^{-3}
$c_{n,sim}^{i}$	simulated variables	kg m^{-3}
$c_{x,f}$	biofilm density	kg m^{-3}
$c_{x,L}$	suspended biomass concentration	kg m^{-3}
$D_{ef,M}$	effective diffusion coefficient of the metal ion in the biofilm	m^2 s-^{1}
E_n	variance between experimental and simulated data	
E_M	removal efficiency of metal ions	%
F_{cr}	circulation flow	m^3 h^{-1}
F_p	back flow	m^3 h^{-1}
F_u	bioreactor inflow	m^3 h^{-1}
h_f	Freundlich isotherm constant for microbial biofilm	
h_L	Freundlich isotherm constant for suspended microbial biomass	
$K_{F,f}$	Freundlich isotherm constant for microbial biofilm	
$K_{F,L}$	Freundlich isotherm constant for suspended microbial biomass	
k_m	mass transfer coefficient in the stagnant liquid layer	mh^{-1}
L_f	biofilm thickness	m
$L_f^{1,2}$	biofilm thickness in the second compartment (*Ni*=2) of the first cascade (*Nl*=1)	m
L_g	stagnant liquid layer thickness	m
m_M	mass of metal ion	kg
$m_{x,f}$	dry weight of microbial biomass in the biofilm	g
$m_{x,L}$	dry weight of suspended microbial biomass	kg
n	bioreactor rotation speed	s^{-1}
n_u	number of observations	
Ni	number of ideally mixed compartments in cascade	
Nl	number of cascades	
Q_m	volumetric removal rate of metal ion	mg L^{-1} h^{-1}
$q_{x,f}$	biofilm adsorption capacity	mg g^{-1}
$q_{x,L}$	suspended biomass adsorption capacity	mg g^{-1}
p	collocation point	
$r_{M,f}$	reaction rate in the biofilm	kg m^{-3} h^{-1}
$r_{M,L}$	reaction rate in the liquid phase	kg m^{-3} h^{-1}
$r_{M,f}^{1,2}$	reaction rate in the second compartment (*Ni*=2) of the first cascade (*Nl*=1) in the biofilm	kg m^{-3} h^{-1}
$r_{M,L}^{1,1}$	reaction rate in the first compartment (*Ni*=1) of the first cascade (*Nl*=1) in the liquid phase	kg m^{-3} h^{-1}
S	inside bioreactor surface	m^2
$S^{1,2}$	the surface for mass transfer in the second compartment (*Ni*=2) of the first cascade (*Nl*=1)	m^2
$S^{Nl,Ni}$	the surface for mass transfer in the ideally mixed compartment of the cascade	$\frac{S}{Nl \cdot Ni}$; m^2
t	time	s
t_R	retention time	h
V_L	liquid volume in the HRTB	m^3

$V_L^{1,1}$	liquid volume in the first compartment (Ni=1) of the first cascade (Nl=1)	m^3
$V_L^{1,2}$	liquid volume in the second compartment (Ni=2) of the first cascade (Nl=1)	m^3
$V_L^{Nl,Ni}$	liquid volume in the ideally mixed compartment of the cascade	$\frac{V_L}{Nl \cdot Ni}$; m^3
z	biofilm depth coordinate	m
z_i	collocation point across biofilm zone parallel to the substrate surface	

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