Iran. J. Environ. Health. Sci. Eng., 2010, Vol. 7, No. 5, pp. 377-384

EFFECT OF HEAVY METALS ON FOULING BEHAVIOR IN MEMBRANE BIOREACTORS

[1]S. Amiri, *[1,2]M. R. Mehrnia, [1]H. Azami, [1]D. Barzegari, [3]M. Shavandi, [1]M. H. Sarrafzadeh

[1]School of Chemical Engineering, College of Engineering, University of Tehran, Tehran, Iran
[2]Oil and Gas Centre of Excellence, University of Tehran, Iran
[3] Department of Biotechnology, Biotechnology Research Center, Research Institute of Petroleum Industry, Tehran, Iran

Received 4 March 2010; revised 30 May 2010; accepted 27 October 2010

ABSTRACT
Presence of heavy metals is considered to be a major challenge in wastewater treatment. In this research the effect of heavy metals such as nickel (Ni), chromium (Cr), and manganese (Mn) on fouling in membrane bioreactors was investigated. Fouling tendency of a cellulose acetate membrane was evaluated in MBRs with different concentrations of the mentioned elements. The concentrations of extractable extracellular polymeric substances in the mixed liquor at the steady-state condition and different concentrations of heavy metals were compared. Also the effects of concentration of heavy metals on mean floc diameter, hydrophobicity, and hence the fouling propensity of membrane was investigated. The Analysis of variance (ANOVA) method has been used to illustrate the important factors for the prediction of the fouling behavior. Results showed that different salts of the same heavy metal ion and various concentrations of heavy metals in wastewater had different effects on sludge properties and hence induced different fouling tendency of sludge.

Key words: Extracellular polymeric substances; Floc size; Fouling; Heavy metals; Membrane bioreactors; Wastewater

INTRODUCTION
Heavy metals are found in wastewater of several industries such as metal finishing, hydrometallurgical, refining, petrochemical, tanneries and battery manufacturing companies. Numerous technologies have been applied for treatment of wastewaters containing heavy metals; however, still significant amount of heavy metals remain in treated effluent. Membrane bioreactor (MBR) is one of the recent advanced technologies that may be utilized for wastewaters containing heavy metals (Mack *et al*., 2004).

MBR consists of an activated sludge system with a membrane filtration. The membrane acts as a barrier that rejects colloidal and suspended matter, thus providing a final effluent of superior quality. This can be proved as an advantage for the removal of metal ions that are attached to suspended solids and colloids. MBR helps better treatment efficiency over the conventional activated sludge process and provides the opportunity for water reuse or reclamation (Stephenson *et al*., 2000). Nevertheless, fouling seems to be the Achilles heel of the submerged MBRs which decreases permeation rate and increases operational costs (Judd, 2004).

Biofouling on membrane surface is unavoidable and caused by the nature of biological system, where microorganisms and bioparticles are the main constituents of biofouling layer (Judd, 2004). Among the parameters of sludge system,

*Corresponding author: E-mail: mmehrnia@ut.ac.ir
Tell:+9821 66 96 77 90/ Fax:+9821 66 95 77 84

extracellular polymeric substance (EPS) content and soluble microbial products have the most important impact on the biofouling (Le-Clech *et al*., 2006). Furthermore, heavy metals are considered as a toxicant group which strongly reduces the activity of microorganisms, changes the sludge culture, and hence induce alterations in EPS, floc size and membrane fouling.

With regard to the chemical aspects, the heavy metals can play a role as coagulants, via charge neutralization, to produce colloidal flocs. If the colloidal flocs become large and dense, they cannot enter the membrane pore, which reduces the pore blocking (Nghiem *et al*., 2005).

Different heavy metals have different effects on the performance of the MBR and this may be accounted in several ways. Firstly, heavy metal-binding sites in biological macromolecules may have intrinsic preferences for some metals, which may explain the differences in toxicity of heavy metals (Albert, 1973). Secondly, metals may be immobilized on sludge by association with different binding sites on the cells membrane and could adsorb asymmetrically on the surface of cells. Some evidence for the preferential binding of heavy metals by activated sludge solids having different particle sizes has been reported (Chen *et al*., 1974). Thirdly, metals may be prevented from binding with sludge particles by association with soluble compounds.

In Fig. 1 the relative response of microorganisms to the presence of heavy metals has been graphically demonstrated quite elegantly by McCarthy (1964). However, a number of different environmental factors, such as pH (Babich and Stotzky, 1977), metal speciation (Sanrdin and Maier, 2002), mixed liquor suspended solids (MLSS) concentration (Stasinakis *et al*., 2002), age of the culture (Stasinakis *et al*., 2002) and presence and concentration of other heavy metals (Josi-Tope and Francis, 1995) can influence the shape of the curve.

Considerable work has been done on the capability of sludge cultures to remove heavy metals confirming the abilities of microorganisms in sludge to remove a variety of heavy metals. Lead, copper and zinc are removed relatively efficiently by activated sludge organisms, whereas chromium (Cr), nickel (Ni), and manganese (Mn) are only partially removed from wastewater using conventional wastewater treatment systems (Chang *et al*., 1995).

Although MBRs has been extensively employed in treatment of different wastewaters containing heavy metals, but less is known about the effects of these elements on the performance and fouling of MBRs. The main objective of this work was to study the effect of sulphate and chloride salts of heavy metals on sludge characteristics and on permeability and fouling of a MBR. Mn, Ni

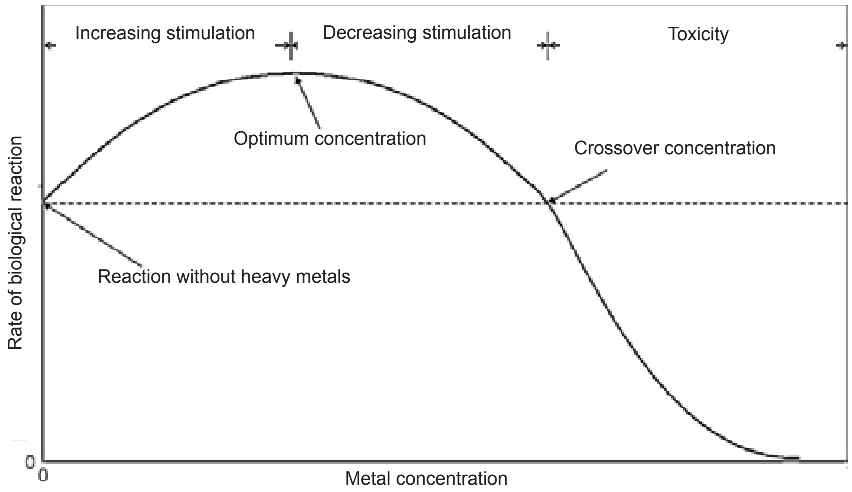


Fig. 1: The effects of heavy metal concentration on microbial growth (adapted from McCarthy, 1964)

and Cr were chosen as important heavy metals because they have been commonly present in municipal and industrial wastewaters (Dilek *et al*., 1998; Stasinakis *et al*., 2002; Stasinakis *et al*., 2003).

Wastewater characteristics, which interact with sludge, also play a key role in membrane fouling. It is important to include the parameters that affect the sludge properties in experiments; such as concerning the influence of bound EPS (TB-EPS), loose bound EPS (LB-EPS), mean floc size, relative hydrophobicity (RH), as negatively charged colloids plays an important role in fouling.

MATERIALS AND METHODS

Membrane bioreactor

The sludge filtration test was conducted in a 1.5 L membrane bioreactor using a 0.45 µm flat sheet membrane filter (Albet-AC-045-47, Spain). The liquid sample was stirred at 400 rpm (Heidolph, MK3001K, Germany).

In each run the stirred cell was filled with 1 L of sample, and a constant vacuum of 0.8 bar was applied for filtration. Hydraulic retention time (HRT) and sludge retention time (SRT) were set at 24 h and 10 d, respectively. Continuous suction was carried out for permeate production. The total permeated flow over 5 h of filtration was measured at ambient temperature and pressure divided by the total surface area expressed in liter per minute per square meter. Mixed liquor temperature of the bioreactor varied slightly between 28°C and 32°C (ambient temperature) throughout the operation. The permeability of the membrane was calculated by the flux rate divided by the transmembrane pressure.

Experimental procedure

The sludge was taken from a petrochemical wastewater plant (Arak petrochemical Co.). The synthetic wastewater included glucose (5 g/L), $(NH_4)_2SO_4$ (3 g/L) and $(NH_4)H_2PO_4$ (1 g/L) and in each experiment just one of the heavy metals with constant concentration was added to this synthetic wastewater.

In order to study the effect of heavy metal concentrations on membrane fouling, 24 hours before each experiment, the sulfate and chloride salts of Ni^{2+} and Mn^{2+} and sulfate salt of Cr^{2+} with concentrations of 0-0.1, 0.2 and 1.0 g/L were added to the sludge in the MBR. In order to adapt mixed culture by heavy metals the sludge was stirred at 250 rpm for 24 h before each experiment. The solid content of samples was adjusted to 4500 mg/L.

EPS extraction and analysis

EPS extraction was performed according to Azami *et al*. (2008) by using formaldehyde and sodium hydroxide. Selection of this method was due to its higher yield of polysaccharide EPSs extraction, which typically constitutes the main portion of EPSs.

Relative hydrophobicity

The relative hydrophobicity of the sludge flocs was measured as adherence to hydrocarbons. To determine the relative hydrophobicity, the method adopted by Chang and Lee (1998) was employed. 40 mL of the activated sludge suspension was homogenized by stirring (1000 rpm for 2 min) at 4 °C to disrupt the flocs to single cells and small micro-colonies. 20 mL pure n-hexadecane was added to the suspension and agitated uniformly for 5 min in a separator funnel. After 30 min relaxation, when the two phases were separated completely, the aqueous phase was transferred into another glass container. The relative hydrophobicity was expressed as the ratio of MLSS concentration in the aqueous phase after emulsification (MLSSe) to the concentration of MLSS in the aqueous phase before emulsification (MLSSi):

Relative hydrophobicity (%) :

$$(1- MLSS_e /MLSS_i) \times 100 \qquad (1)$$

Particle size distribution

The flocs size distribution of the sludge, which might affect the fouling tendency, was examined by light microscopy. The images were captured on a video-microscope (BMZ-04-DZ, Behin Pazhuhesh Eng. Co, Iran) via charged coupled device connected to a computer. The images were taken at different places on slides prepared by sampling method according to Wang and Gregory (2002). The digital photographs were processed and enhanced by using Image Processing Software that enabled to clearly distinguish the floc boundaries. These images provide a two-dimensional representation of the flocs in sludge culture in the MBR. Because of uniform shapes of flocs, an equivalent floc diameter (Feret diameter) was estimated based on the image area and the assumption of spherical shape. The number of measured flocs were chosen according to the British Standard (BS3406, 1984) for microscope counting which suggests that a minimum of 625 particles should be sized in order to get a representative size distribution.

Statistical analysis

The decision, concerning which parameters affect the response of investigated process, are made with Analysis of variance (ANOVA). Analysis of variance will be the predominant statistical method used to interpret experimental data, since this method is most objective (Keppel and Zedeck, 1989). The ANOVA analyses were done with software package "Design expert" V6.0.

RESULTS

This study dealt with the biological aspects of membrane fouling in MBRs containing different concentrations and types of heavy metals.

Fig. 2 presents the membrane permeability versus concentration of $MnSO_4$, $MnCl_2$, $NiSO_4$, $NiCl_2$ and $CrSO_4$.

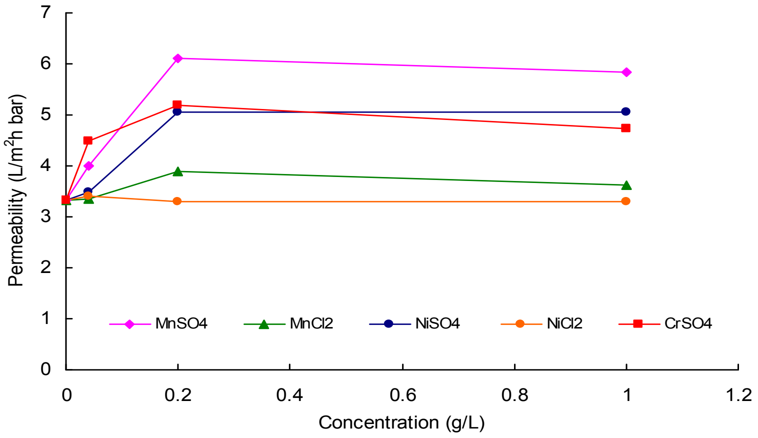


Fig. 2: The membrane permeability versus different concentrations of heavy metals

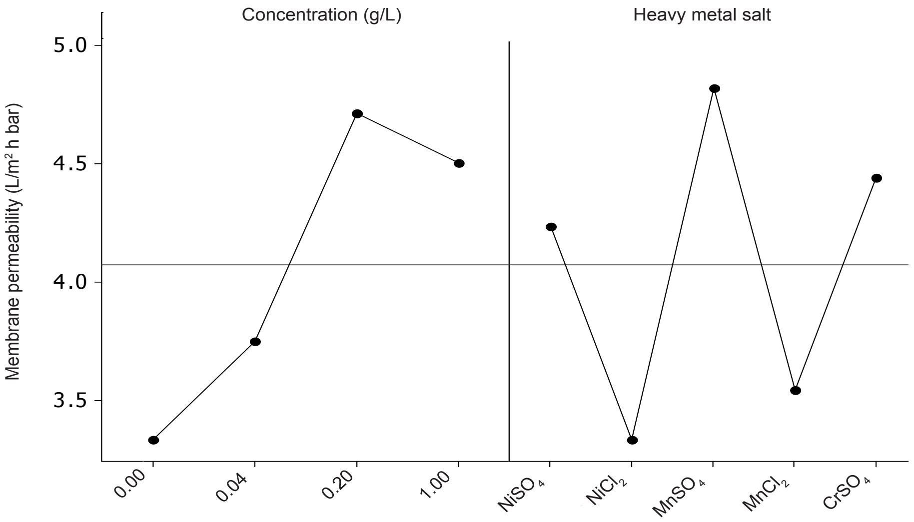


Fig. 3: Univariate subfunctions showing the influence of (left) the kind of heavy metal salt and (right) concentration, on membrane permeability

The univariate terms of the ANOVA analysis shown in Fig. 3 reflect relations between different heavy metal salts and the concentration of heavy metal on the permeability of membrane.

It may be seen that for a given set of inputs, the constructed model selected the kind of heavy metal as the main parameter influencing fouling behavior. However, the influence of the heavy metal varies with different salts.

The results of the analysis of variance are presented in Table 1. As it can be seen, the membrane permeability was affected by the kind of heavy metal and its concentration.

Fig. 4 indicates LB-EPS concentration versus concentration of $MnSO_4$, $NiSO_4$ and $CrSO_4$.

TB-EPSc and the mean floc size versus concentration of heavy metals for $MnSO_4$ and $CrSO_4$ are shown in Fig. 5(a,b). Results indicate that for both $MnSO_4$ and $CrSO_4$ the mean floc diameter and TB-EPS are proportional to heavy metal concentration. The increase in the floc size is simultaneous with increasing of TB-EPSc.

TB-EPS contributes in removing heavy metals, and helps in formations of flocs by promoting microbial aggregation. It has been found that total EPS in a floc represents up to 80% of the mass of the entire sludge (Sobeck and Higgins, 2002). After noticing the nature of bioflocs it can be inferred that interactions between the EPS will be important to bioflocculation along with the characteristics of the EPS.

Ionic exchange phenomena form complexes with negative and positive charged groups, cause the adsorption and precipitation of EPS. As a result the mean floc diameter increases.

Concentrations between 0 to 0.3 g/L of MnSO4 cause the increase in the floc diameter (shown in Fig. 5 a). But for $CrSO_4$ there is an exception at 0.2 g/L and concentrations higher than 0.2 g/L CrSO4 prevent the formation of larger flocs in the MBR (Fig. 5 b).

Floc size has a direct role in the permeability by affecting the porosity of cake layer on the membrane. Besides, as indicated in Fig. 2, the

Table 1: Summary of the results obtained by the ANOVA analysis used for fouling behavior

Source	Degree of freedom	Sum of square error (SS)	Adjusted SS	Adjusted mean SS	F	P
Heavy metal salt	4	6.1768	6.1768	1.5442	4.48	0.019
Concentration (g/L)	3	6.2184	6.2184	2.0728	6.01	0.010
Error	12	4.1369	4.1369	0.3447		
Total	19	16.5320				
S = 0.587149		R-Sq = 74.98%		R-Sq(adj) = 60.38%		

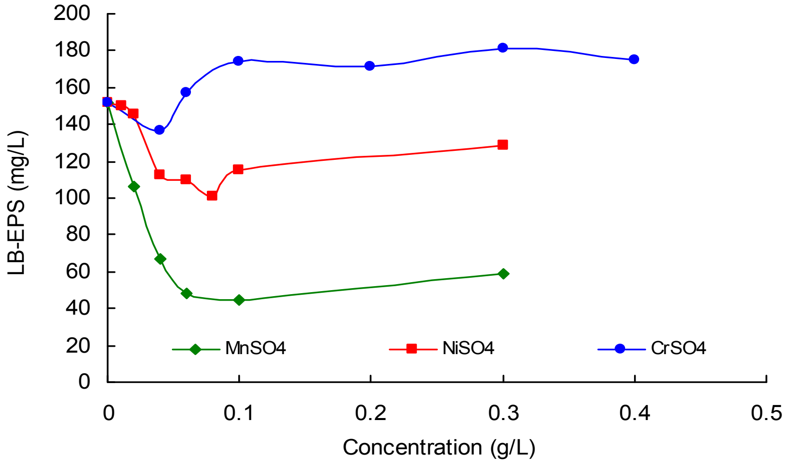


Fig. 4: LB-EPS concentration for different concentrations of heavy metals

permeability of MBR (containing 0.3 g/L $CrSO_4$) is lower than the MBR with concentration of 0.2 g/L $CrSO_4$.

In Fig. 6, RH percentage of sludge versus concentration of heavy metals is shown. It indicates that the RH of the sludge decreases by increasing concentration of heavy metals.

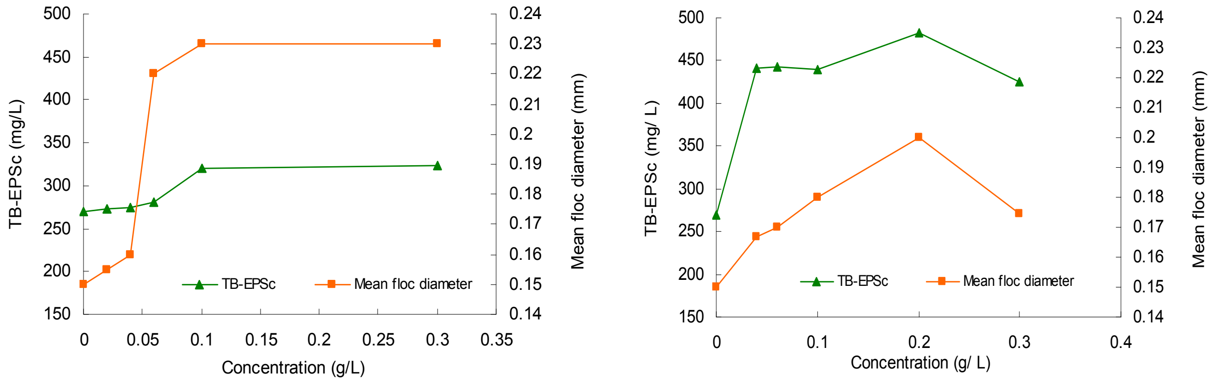
The nature of the cellulose acetate membrane is hydrophilic. In the range of 0.01 to 0.06 g/L of heavy metals, $NiCl_2$ causes the most RH reduction of the sludge which causes the minimum membrane permeability due to increased affinity of the mixed culture to the membrane surface.

In the range of 0.01 to 0.06 g/L MnSO4 and $CrSO_4$, RH is independent of concentration (Fig. 6); but the permeability is assumed to be dependent on the cake layer porosity that increases due to better flocculation of sludge caused by heavy metals bridges.

Another comparison between $NiSO_4$ and $CrSO_4$ shows that the LB-EPS for $NiSO_4$ is lower than $CrSO_4$ (depicted in Fig. 4). Likewise the $CrSO_4$ permeability is more than $NiSO_4$. This occurrence could be the consequence of other parameters such as sludge hydrophobicity that was shown in Fig. 6. In addition the decrease of sludge RH for $NiSO_4$ was more than $CrSO_4$. Hence, the RH and LB-EPS of sludge play an important role on difference between membrane permeability for $NiSO_4$ and $CrSO_4$.

DISCUSSION

As shown in Fig. 2, it was observed that the efflux increases in the presence of low heavy metal concentrations, nevertheless increasing heavy metal concentrations reduced the increasing rate of permeability. This can be due to the toxic effect of heavy metals on sludge



(a) (b)

Fig. 5: TB-EPSc concentration and the mean floc diameter versus different concentrations of $MnSO_4$ (a) and $CrSO_4$ (b)

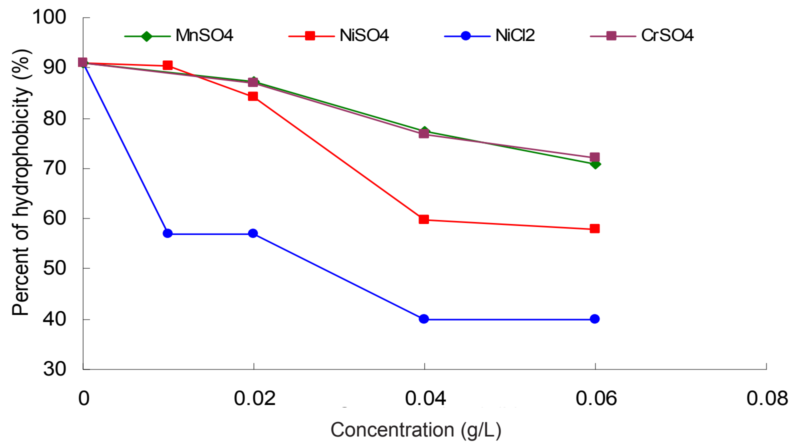


Fig. 6: Relative hydrophobicity of sludge versus concentration of different heavy metals

properties such as EPS concentration at heavy metal concentrations higher than 0.2 g/L and hence declining the membrane permeability. The results indicate that the membrane permeability for $MnSO_4$ and $NiSO_4$ is more than $MnCl_2$ and $NiCl_2$, respectively. This could be due to toxic effect of chloride anions on sludge culture. Furthermore, Mn is on seventh group of periodic table and has bigger atomic radius (164 pm) than Ni (in tenth group, 149 pm atomic radius). Hence, membrane has more permeability with Mn^{2+} containing sludge than Ni^{2+} due to higher ability to form complex with cellular materials. For the same reason Cr^{2+} (Cr is in sixth group of periodic table) at low concentration in sludge has larger permeability than Mn^{2+}. While at higher concentrations the membrane permeability in the presence of $MnSO_4$ is more than that of $CrSO_4$ which could be due to more toxicity of $CrSO_4$ on sludge properties at high concentrations.

Based on the results shown in Fig. 4, it was shown that with increasing the concentration of heavy metals up to about 0.1 g/L, the LB-EPS concentration decreases. While by increasing the concentration of heavy metals from 0.1 to 0.4 g/L LB-EPS increases. The LB-EPS concentration for $MnSO_4$ is less than that for other heavy metals. Lower LB-EPS concentration results in higher membrane permeability as shown in Fig. 2. This behavior is the result of the fact that metal toxicity is mainly dependent on the metal concentration, speciation, the state of microbial growth and the biomass concentration as reported by Arican and Yetis (2002). Microbial resistance to heavy metal is mediated by a series of systems like the permeability barrier, intra- and extra-cellular sequestration, active transport efflux pump, enzymatic detoxification and reduced sensitivity of cellular targets to metal ions (Tanaka *et al*., 2004; Nies, 2003). The forgoing mechanisms increase the LB-EPS concentration.

This first version of our empirical model of membrane permeability indicates that this type of approach might help to better classify the factors influencing membrane fouling. If it is possible to give reasonably accurate fouling behavior with a simple method, it will also be easier to convince the researchers to take the possible important factors on fouling. Future work will therefore try to improve the model and extend it to other MBRs containing different kind of wastewaters.

The LB-EPS, TB-EPS, mean floc size and sludge RH are significant parameters that affect membrane fouling. This study shows that different heavy metals have specific effect on sludge properties and membrane fouling; this may be due to their distinct toxicity limit and different intrinsic preferences of heavy metal-binding sites of biological macromolecules.

It could be a useful tool in fouling behavior studies to demonstrate interactions that determine membrane permeability decreases and to assess the potential to reduce membrane fouling with different options in MBRs.

The results also show that presence of low concentration of heavy metals in the MBR system results in lower LB-EPS and higher TB-EPS hence reduce fouling and increase the membrane permeability. Future research will be directed towards deepening knowledge on mechanisms of metal interactions with biological macromolecules and its effect on fouling in MBR systems.

ACKNOWLEDGEMENTS

Authors present their sincere thanks to Research and Technology Management of NIORDC for financial support.

REFRENCES

Albert, A., (1973). Selective toxicity. Chapman and Hall, London, UK.

Arican, B., Gokcay, C. F., Yetis, U., (2002). Mechanistics of nickel sorption by activated sludge. Process Biochem., **37**: 1307–1315.

Azami, H., Mehrnia, M.R., Sarrafzadeh, M.H., Kazemzadeh, M., Mafirad, S., Mostoufi, N., (2008). Membrane biofouling by soluble microbial products in a membrane bioreactor, 18th international congress of chemical and process engineering, Prague, Czech Republic.

Babich, H., and Stotzky, G., (1977). Sensitivity of various bacteria including actinomycetes and fungi to cadmium and the influence of pH on sensitivity, Appl. Environ. Microb., **33**(3): 681–695.

BS3406 – British standard methods for determinination of particle size distribution (1984).

Chang, D., Fukushi, K., Ghosh, S., (1995). Simulation of activated sludge cultures for enhanced heavy metals removal. Water Environ. Res., **67**: 822–827.

Chang, I., and Lee, C., (1998). Membrane filtration characteristics in membrane coupled activated sludge system the effect of physiological states of activated sludge on membrane fouling. Desalination, **120**: 221–233.

Chen, K.Y., Young, C.S.R., Rohatgi, N., (1974). Trace metals in waste water effluents. J. Water Poll. Control Fed., **46**: 2663-2675.

Dilek, F.B., Gokcay, C.F., Yetis, U., (1998). Combined effects of Ni(II) and Cr(VI) on activated sludge. Water Res, **32**(2): 303-312.

Josi-Tope, G., and Francis, A.J., (1995). Mechanisms of biodegradation of metal-citrate complexes by *Pseudomonas fluorescens*. J. Bacter., **177**:1989–1993.

Judd, S., (2004). A review of fouling of membrane bioreactors in sewage treatment, Water Sci. Tech., **49**: 229-35.

Keppel, G., and Zedeck , S., (1989). Data Analysis for Research Designs: Analysis of Variance and Multiple Research/Correlation Approaches, W.H. Freeman, New York, USA.

Le-Clech, P., Chen, V., Fane, T.A.G., (2006). Fouling in membrane bioreactors used in wastewater treatment, J.Mem.Sci., **284**:17–53.

Lee, W., Kang, S., Shin, H., (2003). Sludge characteristics and their contribution to microfiltration in submerged membrane bioreactors. J. Mem. Sci., **216**: 217- 227.

Mack, C., Burgess, J. E., Duncan, J. R., (2004). Membrane bioreactors for metal recovery from wastewater: A review, Water SA, **30**(4): 521-532.

McCarthy, P.L., (1964). Anaerobic waste treatment fundamentals, part III. Toxic materials and their control, PublicWorks, **95**(11): 91–94.

Nies, D.H., (2003). Efflux-mediated heavy metal resistance in prokaryotes. *FEMS* Microb. Rev., **27**: 313–339.

Nghiem, L.D., Oschmann, N., Schäfer, A.I., (2005). Grey water recycling by direct ultrafiltration: understanding fouling. In: The Proceeding of the Integrated Concepts in Water Recycling (ICWR2005), 14–17 February, Wollongong, Australia.

Sanrdin, T.R., and Maier, R.M., (2002). Effect of pH on cadmium toxicity, speciation, and accumulation during naphthalene biodegradation. Environ. Toxic. Chem., **21**(10): 2075–2079.

Sobeck, D.C., and Higgins, M.J.,(2002). Examination of three theories for mechanisms of cation-induced bioflocculation. Water Res, **36**: 527–538.

Stasinakis, A.S., Mamais, D., Thomaidis, N.S., Lekkas, T.D., (2002). Effect of chromium (VI) on bacterial kinetics of heterotrophic biomass of activated sludge. Water Res, **36**: 3341–3349.

Stasinakis, A.S., Thomaidis, N.S., Mamais, D., Karivali, M., Lekkas, T.D., (2003). Chromium species behaviour in the activated sludge process, Chemosphere, **52**, 1059–1067.

Stephenson, T., Judd, S.J., Jefferson, B., Brindle, K., (2000). Membrane bioreactors for wastewater treatment. IWA Publishing, London, UK, pp. 6-7.

Tanaka, Y., Tsumoto, K., Nakanishi, T., Yasutake, Y., Sakai, N., Yao, M., Tanaka, I., Kumagai, I., (2004). Structural implications for heavy metal-induced reversible assembly and aggregation of a protein: the case of Pyrococcus horikoshii CutA. FEBS Lett., **556**:167–174.

Wang, X.C., and Gregory, J., (2002). Structure of Al-Humic Flocs and their Removal at Slightly Acidic and Neutral pH. Water Sci. Tech., **2**(2): 99-106.