Treatment of inhibitory phenolic compounds in membrane bioreactor

Q. T. T. Thuy* and C. Visvanathan**

** Environmental Engineering Course, Department of Urban Engineering, University of Tokyo, 7-3-1, Hongo, Bunkyo, Tokyo 113-865, Japan. Email: qthuy@env.t.u-tokyo.ac.jp*
***Environmental Engineering and Management Program, School of Environment, Resources and Development, Asian Institute of Technology, Pathumthani, 12120, Thailand. Email: visu@ait.ac.th*

Abstract The treatability of toxic inhibitory phenolic compounds was investigated using membrane bioreactor system with an innovative design. Initially, the system was fed with phenol (500 mg/L) followed by addition of 2, 4-Dichlorophenol, to study the effect of toxic compound on the system. Phenol, 2,4-Dichlorophenol (2,4-DCP), TOC and COD removal were found to be higher than 98.99% when the organic load ranged between 1.80 to 5.76 kg/m^3.d COD. Bound and soluble extracellular polymeric substances (EPS) production in sludge was found to be 37.6-47 mg C/g VSS and 0.85-2.48 mg C/g VSS, respectively. Further, when the relationship between sludge properties and EPS components was studied, it was found that settleability had no correlation with EPS directly, though it was correlated to protein/carbohydrate (P/C) ratio.

Keywords Biokinetic studies; 2,4-Dichlorophenol; extracellular polymeric substances; membrane bioreactor; phenol; sludge properties

Introduction

The presence of toxic phenolic compounds such as Phenol and 2,4-Dichlorophenol in wastewater is found in industries such as wood processing, pharmaceutical, pesticide and petrochemical plants. Treatment of such inhibitory phenolic compounds have been studied using sequencing batch reactors (SBR) (Young and Lant, 2001), SBR coupled with granular activated carbon (Buitron et al., 2001), etc. However, difficulties faced in conventional biological treatment processes to treat toxic phenolic wastewater mainly include sludge deflocculation (Galli *et al.*, 1998) and instability due to inhibitory properties of phenolic compounds to the microorganisms, in order to meet effluent discharge standards.

Membrane bioreactors (MBR) in recent years have been used for varied applications. It has many advantages such as high quality effluent, small size of treatment unit, less sludge production, flexibility in operation (Visvanathan *et al.*, 2000), higher volumetric loading and high stability in addition to high biomass retention. Though, studies have been carried out in varied application of membrane bioreactor, little has been studied on the treatment of difficult to biodegrade industrial wastewater such as phenolic wastewater.

Apart from the treatment efficiency, the other important aspects in MBR treatment are the growth kinetics, membrane fouling and the sludge properties. The effectiveness of any biological treatment is usually governed by the growth of microorganisms. The treatment of the pollutants takes place in a series of oxidation/reduction reactions, which are catalyzed by microorganisms through the production of enzymes. Hence, biokinetic study that indicates the effectiveness of the microorganisms to degrade organic compounds is important for environmental engineers. The rate at which the organic compounds are removed is dependent on the biomass (measured as MLVSS) present in the treatment process and rate at which the microorganisms utilize these organic substrates to degrade it into harmless products. However, the rates of biomass production and substrate utilization are interrelated. Biokinetic studies helps in measuring the growth model and coefficients

such as maximum growth rate (μ_{max}), half-velocity constant (K_s) and observed yield coefficient (Y_{obs}). Based on these data and mass balance modeling, the relationship between biomass and substrate can be systematically and quantitatively established for operation and design of treatment processes.

The present research work focuses on treatment effeciency of membrane bioreactor system to treat synthetic wastewater containing phenolic compounds (phenol and 2,4-DCP). A series of biokinetic experiments was also done to obtain the microbial growth pattern of the acclimatised sludge. In addition to the removal effeciency, parameters affecting the membrane biofouling and sludge charecteristics were investigated.

Methods

Feed wastewater

In the present study, a synthetic wastewater containing 500 mg/L Phenol as carbon source was used as a feed, followed by addition of 50 and 100 mg/L 2,4-DCP, consequently. Nutrient salts were added to the feed wastewater to enhance microbial growth. The feed wastewater nutrient composition as follows (all the parameters in mg/L): $(NH_4)_2SO_4$- 270, KH_2PO_4- 300, K_2HPO_4- 200, $NaHCO_3$- 330, $MgSO_4.7H_20$- 500, $MnSO_4.H_2O$- 5, $CaCl_2$- 4, $FeCl_3.6H_2O$- 5, $CuSO_4$- 0.2, $ZnSO_4$ - 0.1, $(NH_4)_6Mo_{24}.4H_2O$- 0.1 and $CoCl_2$- 0.1. Phosphate buffer was used to maintain the pH around 7.

Chemical and equipments

All chemicals used in the study were of analytical grade. U-shaped hollow fiber microfiltration (0.1μm) membrane manufactured by Misubishi Rayon Company, Japan (Sterapore) was used for the study. Phenol and 2,4-DCP were analyzed using gas chromatography (Shimadzu GC-15A) with the column (HP1, part number 19091Z-212, column ID 32 nm), nitrogen as carrier gas and flame ionization detector. TOC was analyzed using Shimadzu TOC-V_{CSN} analyzer.

Experimental set-up

The experimental set-up is presented in Figure 1. Here, the reactor with working volume of 5 L was designed with two chambers (I and II) separated by means of a perforated baffle. The influent entered the reactor in chamber I and the membrane was kept in chamber II. The feed wastewater from mixing tank was pumped to feed tank, from where it was gravity fed to level control tank. The reactor was continuously aerated by compressed air through stone diffusers placed at bottom of chamber I and II. The filtration cycle of 25 min suction, 3 min air backwash at a pressure of 2 Bar and 1 min pressure release was maintained in the reactor with the help of timer and solenoid valves. Activated sludge from an aeration tank of an industrial wastewater treatment plant was used as microbial seed. The acclimatization was done by fill-and-draw process by step-wise increasing the phenol concentration from 30 to 500 mg/L.

The biokinetic studies were carried out in a respirometer with working volume of 0.9L. The respirometer was continuously monitored using an online DO meter. Acclimated sludge was used for the experiment after dilution with mineral medium consisting of 500 mg/L $NaHCO_3$, 15 mg/L N NH_4Cl and 3 mg/L KH_2PO_4. Phenol was prepared as concentrated substrate and injected into the cell through the expansion funnel with a syringe. The initial substrate concentration to initial biomass concentration taken for the study was around 0.01-0.2 (Mathieu and Etienne, 2000). All the experiments were conducted at temperature of 20 ± 1^oC using water jacket. pH of the system was maintained at 7.0 ± 0.2 using HCl and Na_2CO_3 solution. 10 mg/L of Allylthiourea (ATU) was used to inhibit nitrification. The sludge after dilution was aerated for at least 2 hours until it reached its endogenous phase before starting up the experiment. Oxygen uptake rate (OUR) of endogenous phase was recorded (OURx,e). Later on, an accurate amount of concentrated substrate was added to

obtain the desired So/Xo (0.01-0.2) and total OUR of the system was recorded (OURx, t). Aeration was necessary when the DO concentration reduced below 2 mg/L. The DO was continuously monitored using DO meter connected to a computer.

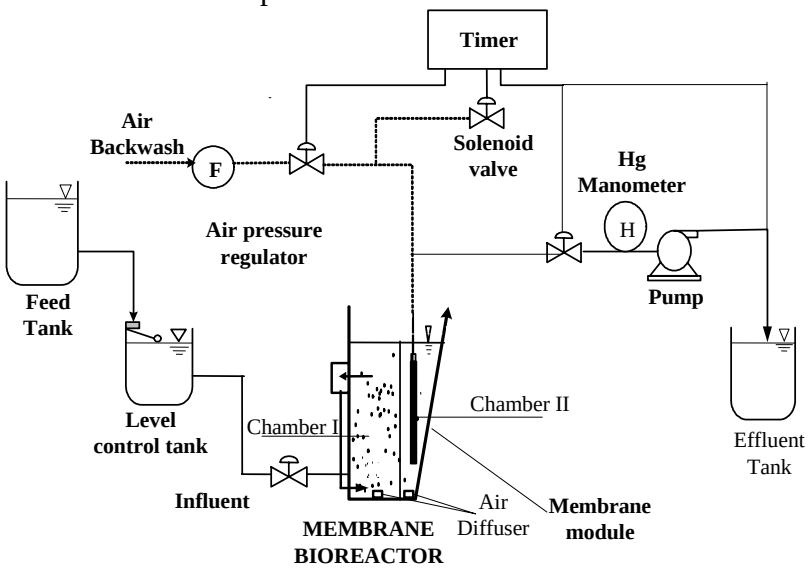


Figure 1 Experimental set-up

Based on the results obtained, the endogenous respiration (OURx,e), total respiration rate (OURx,t) and net oxygen consumption (OC) were calculated. Consequently, specific rate (OURx, ox) of substrate was calculated using the formulae:

$$OURx,ox = OURt - OURx,e \qquad \text{Equation 1}$$

Specific rate of substrate removal at substrate concentration S is given by:

$$r_x = \frac{r_{X,OX}}{OC/S} \qquad \text{Equation 2}$$

OC/S is the mass of oxygen utilized by the biomass to degrade the readily biodegradable substrate (S) and is measured by:

$$\frac{OC}{S} = 1 - fY \qquad \text{Equation 3}$$

where F= COD/VSS ratio of the sludge.

Specific growth rate is expressed as

$$\mu = Y.r_x \qquad \text{Equation 4}$$

Applying equations 1 to 4, specific growth rate (μ) and yield coefficient (Y) were calculated. Regression analysis gave the model kinetics for bacterial growth, half velocity constants (Ks) and observed specific growth rate (μ_{obs}).

The study investigated the efficiency of MBR in terms of permeate quality, membrane fouling and system stability under different HRTs. The system was operated with two phases: 1) Run I: 500 mg/L phenol, 2) Run II: 500 mg/L phenol with 50 and 100 mg/L 2,4-DCP, used sequentially. Effluent quality parameters such as COD, phenol and 2,4-DCP, TOC, MLSS, TMP, DO and pH were measured. Sludge characteristics were analyzed in terms of viscosity, settleability measured as sludge volume index (SVI), EPS (soluble and bound), protein, carbohydrate and dewaterability measured as capillary suction time (CST).

Analytical methods

Phenol and 2,4-DCP were analyzed using gas chromatography with flame ionization detector. While measuring the EPS, the bound and soluble EPS present in the bioreactor were separated by centrifuging mixed liquor at 3,200 rpm for 30 minutes. As the supernatant represents the soluble EPS, it was analyzed as TOC (mg C/gVSS). The centrifuged sludge was washed and re-suspended with NaCl (0.9%), then heated at 100ºC for 1 hour. Bound EPS was separated by centrifugation at 3,200 rpm for 30 minutes (Chang and Lee, 1998) and measured as TOC. The protein and carbohydrate component of both bound and soluble EPS were examined. Other analyses in the study were conducted according to the procedure given in Standard Methods (APHA *et al.*, 1998).

Results and Discussion

Biokinetic study

The biokinetic parameters of the membrane bioreactor sludge were measured in order to study the growth kinetics of the sludge under toxic condition. Figure 2 presents the relationship between specific growth rate and substrate concentration. The growth curve of the biomass followed Monad's kinetic growth pattern with μ_{max}, Y_{obs} and K_s of 8.86 d^{-1}, 0.32 and 18 mg/L, respectively. In conventional activated sludge process, the typical value of μ_{max} of the sludge is 6.0 d^{-1}, Y_{obs} is 0.4 and K_s is 20 mg/L at a maximum substrate removal rate 15 g COD/ g VSS.d (Henze *et al.*, 1987;Barker and Dold, 1997).

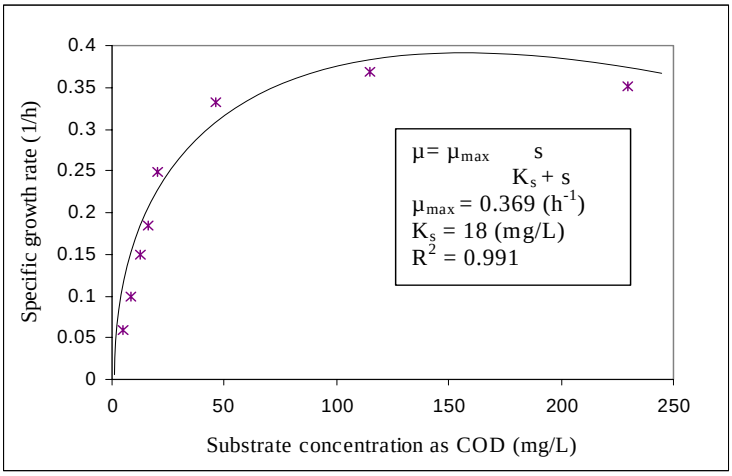


Figure 2 Growth of membrane bioreactor sludge at different substrate concentration

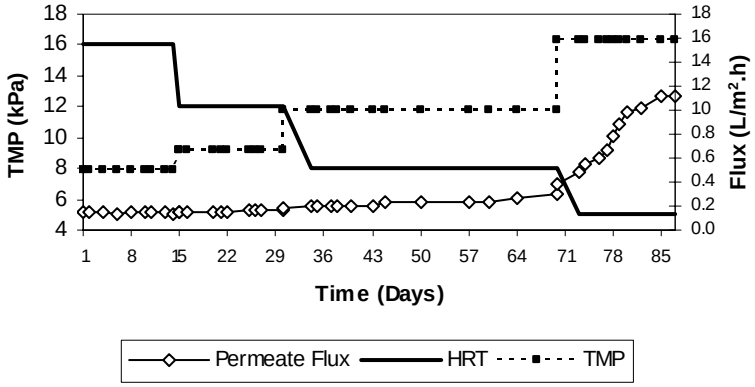
It is interesting to note that μ_{max} and substrate utilization rate of present membrane bioreactor sludge was found to be higher than that of conventional activated sludge. However, Y_{obs} was found to be lower, which suggests that the portion of substrate utilized for the production of new cells in MBR was lower than the conventional process. The reason for low yield coefficient in comparison with the conventional process could be the long SRT (20 days) in the MBR systems, during which endogenous respiration takes place.

The maximum substrate removal rate of bioreactor sludge was 27.7 g COD/ g VSS.d, which was also found to be relatively high compared with conventional treatment process. However, Rittmann and McCarty, (2001) found heterotrophs could have a maximum substrate removal rate of 27 g BOD/ g VSS.d in activated sludge. Thus, through the biokinetic study, it could be concluded that

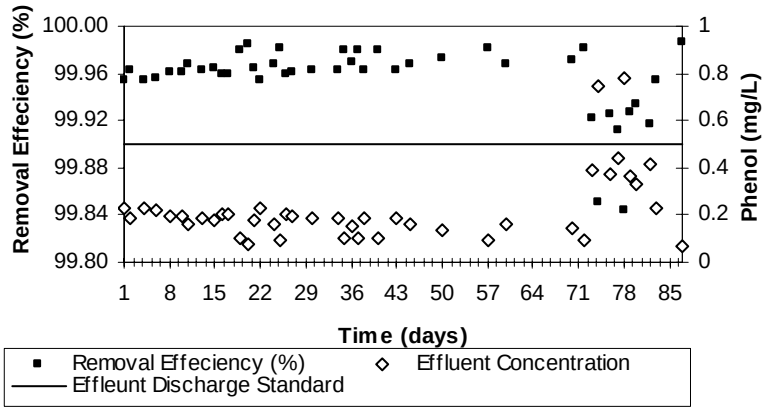
acclimatization was effective and the sludge used in the study was well adapted to treat toxic inhibitory wastewater.

Treatment efficiency

In Run I, the treatment efficiency of MBR in the removal of 500mg/L phenol was studied at different HRTs (16, 12, 8 and 5h). The results obtained are presented in Figure 3. Phenol, TOC and COD removal was found to be more than 98.99% when the organic loading was varied from 1.80 to 5.76 kg/m^3.d COD. The treatment efficiency of the MBR system did not vary significantly while varying the HRT. Thus, it could infer that MBR system can be well suited for treating wastewater with high concentration of phenol.



(a)



(b)

Figure 3 Removal efficiency of MBR systems at different HRT: a) TMP and flux variation; b) Phenol removal efficiency (influent 500 mg/L phenol, 1200 mg/L COD, 374 mg/L TOC)

As the wastewater with Phenol could be treated effectively using a MBR system, the study was further continued with a more toxic compound, namely the 2,4-DCP (Run II). In order to further investigate the stability of MBR system, two concentrations (50 and 100 mg/L) of 2,4-DCP was added to feed water with Phenol. HRT and SRT of the system were 8h and 20 days, respectively. The results showed that around 95% of 2,4-DCP and 99% of phenol could be removed. The TOC removal effeciency was found to be 95%. The transmembrane pressures of the membrane did not change upto 32 days. This confirms that the MBR system was efficient in treating toxic wastewater containing a mixture of compounds.

Sludge characteristic study

Membrane fouling is a common problem found in membrane bioreactors. Recent studies have focused on EPS as a major foulant causing membrane biofouling contributing to the decreased permeate flux. Though the major focus is towards bound EPS, little importance has been given to the EPS components (protein, carbohydrate) and its soluble form, which may to a large extent also affect membrane fouling. Here, changes of EPS production, composition and accumulation with F/M ratio and operating time were investigated. Table 1 summarizes the experimental data of the three samples taken at a different periods of membrane operation.

Table 1 Characteristics of membrane bioreactor sludge

Characteristics		Sample I	Sample II	Sample III
Operation time (days)		30	70	90
SVI (mL/L)		68	53	77
CST (s)		25	39	101
Viscosity (mPa.s)		15	26	31
F/M		0.23	0.40	0.25
Soluble EPS	Soluble EPS (mg C/gVSS)	0.85	1.55	2.48
	Protein/Carbohydrate	0.61	0.47	0.19
Bound EPS	Bound EPS (mg C/gVSS)	37.60	47.08	39.85
	Protein/Carbohydrate	0.97	2.72	1.75

EPS production depends on environmental conditions such as carbon source, organic loading, bacterial culture, nutrients, etc. As Phenol and 2,4-DCP are toxic and inhibitory compounds, it was expected that the bound EPS produced by the microorganisms will function as a protective barrier and help in accumulating the compounds, subsequently degrading them. However, as summarized in Table 2, the bound EPS production did not change significantly with change in F/M ratio. Further, as indicated by biokinetic studies, the microbial populations were found to be well adapted for the degradation of phenol. Thus, it could be said that bound EPS production in the suspended microbial growth is independent of F/M ratio and HRT.

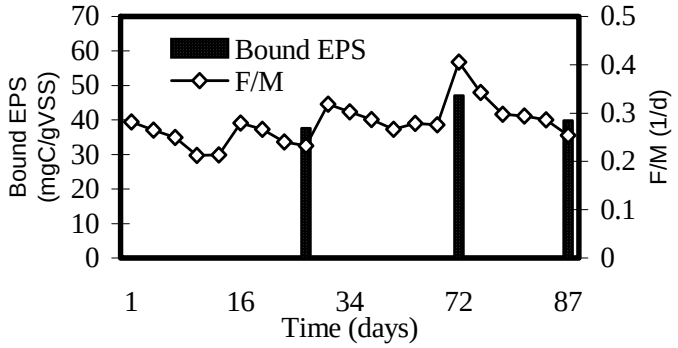
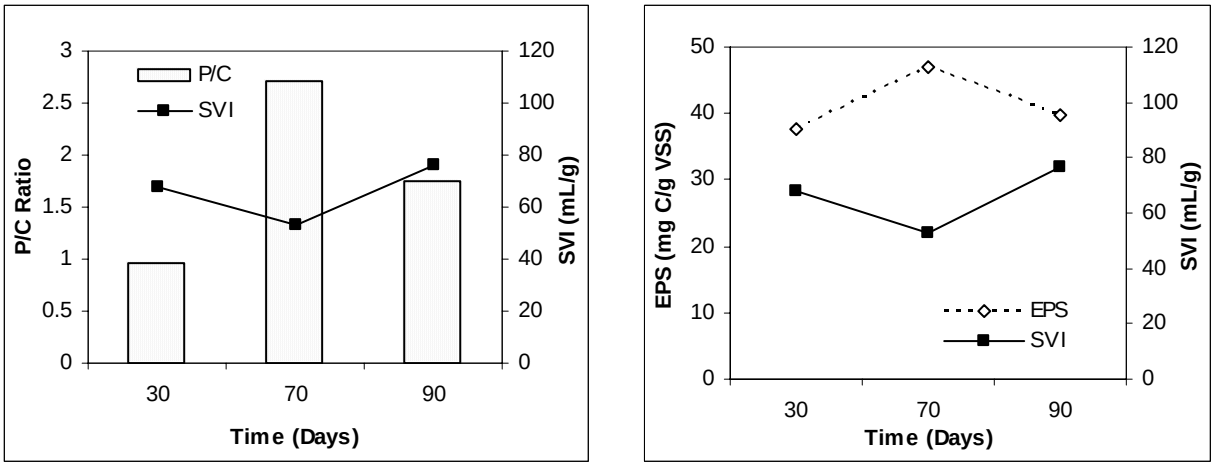


Figure 4 F/M and bound EPS production

EPS act as the bridge to aggregate bacterial cells and create floc/biofilms. The presence of EPS is known to enhance the settleability of the sludge and thus is advantageous in conventional activated sludge process, though it is not so in the membrane coupled activated sludge process due to its role in cake layer formation. The driving forces of the formation are the sludge surface charge and hydrophobicity. However, total EPS content in sludge does not affect hydrophobicity and surface charge of sludge (Urbain *et al.*, 1992). Therefore, while establishing the relationship between proteins to carbohydrate ratio (P/C) of the bound EPS and SVI, it was found that they were inversely

proportional. Figure 4 signifies the proportionality between P/C and SVI, not EPS and SVI. Carbohydrate is usually found to have a negative influence on the hydrophobicity, while protein has a positive influence on the hydrophobicity of the sludge. Thus, the sludge with higher P/C in bound EPS were less negatively charge and more hydrophobic, and resulted in better sludge settleability (lower SVI) in activated sludge process and stronger cake layer on membrane systems. Hence, it can be concluded that the P/C of bound EPS plays a major role in sludge settleability and also acts as major factor in membrane fouling.



(a) **(b)**

Figure 5 (a) P/C ratio and SVI correlation; (b) SVI and EPS

Soluble EPS was found to gradually increase with operating time. The soluble EPS, in the mixed liquor measured as TOC, is actually the soluble microbial product (SMP) of phenol degradation. SMP mainly consists of high-molecular weight organic compounds. Thus, the accumulation of soluble EPS within the reactor was caused by membrane interception performance (Figure 6). Some researches have reported that SMP is considered as the major foulant in MBR due to its gel formation in membrane pore (Nagaoka *et al.*, 1996;Chang and Lee, 1998). Figure 7 shows the increasing trend of soluble EPS production along with its protein and carbohydrate component in the mixed liquor. The rate of increase in carbohydrate was found to be greater than that of the protein. Huang *et al.*, 2002 investigated the behavior of SMP in MBR and found that the SMP in the supernatant was accumulated and degraded after 5 months. As carbohydrate degrades faster, the protein in the soluble EPS could act as a major factor affecting membrane fouling in comparison to the carbohydrate.

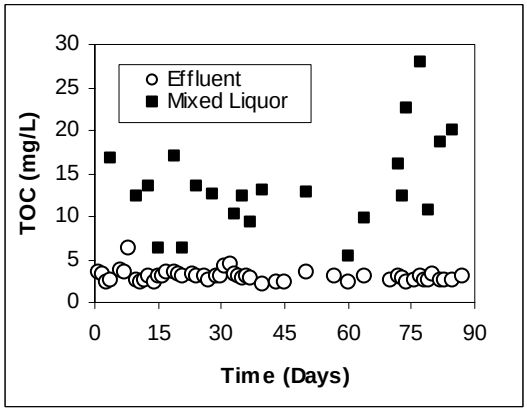


Figure 6 Effluent and mixed liquor TOC

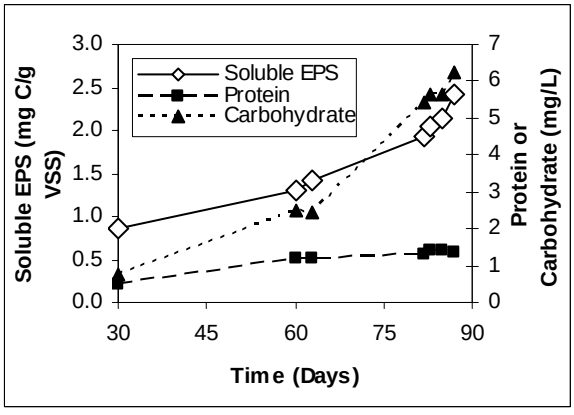


Figure 7 Soluble EPS and its components

Conclusions

Biokinetic coefficients of the sludge taken from membrane bioreactor while treating phenolic wastewater were found to be having a μ_{max} of 0.369 h^{-1}, K_s of 18 mg/L and Y_{obs} of 0.32 d^{-1}. This suggests that the sludge in membrane systems is effective in phenolic wastewater treatment. This was further confirmed by the treatment efficiency of membrane bioreactor in phenol and 2,4-DCP removal. The present study shows that the membrane bioreactor systems are effective in toxic phenolic wastewater treatment. When phenol and 2,4-DCP were used as a carbon source in MBR system, 98.99% of phenol, 2,4-DCP, TOC and COD removal could be obtained when organic loading was increased from 1.80 to 5.76 kg/m^3.d COD. The reactor design used in the study played a significant role in reducing membrane fouling. Bound EPS production in the sludge, which is known to enhance membrane biofouling, was found to be independent of F/M ratio. Instead, P/C ratio in the bound EPS was found to play a major role in sludge settleability and membrane fouling. Soluble EPS production of sludge in the MBR increased with time period of membrane operation. Similar to bound EPS, the P/C ratio of the soluble EPS played a role in membrane fouling rather than individual components of EPS.

Acknowledgement- This work was supported by Swedish International Development Cooperation (SIDA) and Southeast Asian Center for Water Environmental Technology (SACWET).

References

APHA, AWWA and WPCF. *Standard methods for the examination of water and wastewater*, 20th Edition. Washington DC, USA (1998).

Barker, P.L. and Dold, P.L. (1997). General model for biological nutrient removal in activated sludge systems: model presentation. *Wat. Environ. Res.,* **69**(5), 969-984.

Buitron, G., Soto, G., Vite, G. and Moreno, J. (2001). Strategies to enhance the biodegradation of toxic compounds using discontinuous processes. *Wat. Sci. Tech.,* **43**(3), 283-290.

Chang, S.I. and Lee, C.H. (1998). Membrane filtration characteristics in membrane coupled activated sludge system-The effects of physiological states of activated sludge on membrane fouling. *Desalination,* **120**, 221-223.

Galli, N.I., Mittelman, A.S. and Zohar, O.S. (1998). Biomass deflocculation and process disturbances exerted by phenol induced transient load conditions. *Wat. Sci. Tech.,* **38**(8-9), 105-112.

Henze, M., Grady, C.P.L., Gujer, W., Marais, R. and Matsuo, T. (1987) In *IAWPRC Scientific and Technical Reports, no. 1,* London.

Huang**,** X., Liu, R. and Qian, Y. (2002). Behaviour of soluble microbial products in a membrane bioreactor. *Process Biochemistry,* **36**(5), 401-406.

Mathieu, S. and Etienne, P. (2000). Estimation of wastewater biodegradable COD fractions by combining respirometric experiments in various So/Xo ratios. *Wat. Res.,* **34**(4), 1233-1246.

Nagaoka**,** H., Ueda, S. and Miya, A. (1996). Influence of bacterial extracellular polymers on membrane separation activated sludge process. *Wat. Sci. Tech.,* **34**(9), 165-172.

Rittmann, B.E. and McCarty, L.P. (2001) *Environmental Biotechnology: Principles and Applications,* McGraw-Hill, New York.

Urbain, V., Block, J.C. and Manem, J. (1992). Bioflocculation in activated sludge, an analytical approach. *Wat. Sci. Tech.,* **25**(4-5), 441-443.

Visvanathan, C., Ben Aim, R. and Parameshwaran. (2000). Membrane separation bioreactors for wastewater treatment. *Environ. Sci. Tech.,* **30**(1), 1-48.

Young, E.T. and Lant, P.A. (2001). Biodegradation of high strength phenolic wastewater using SBR. *Wat. Sci. Tech.,* **43**(3), 229-306.