Desalination and Water Treatment
www.deswater.com

doi: 10.1080/19443994.2013.793477

51 (2013) 6940–6951
November

Taylor & Francis
Taylor & Francis Group

Biofouling mitigation using *Piper betle* extract in ultrafiltration MBR

M.F. Siddiqui[a], L. Singh[b], A.W. Zularisam[c,*], M. Sakinah[a]

[a]*Faculty of Chemical & Natural Resources Engineering, Universiti Malaysia Pahang, Gambang, Kuantan 25000, Malaysia*
[b]*Faculty of Industrial Sciences & Technology, Universiti Malaysia Pahang (UMP), Lebuhraya Tun Razak, Gambang, Kuantan 26300, Malaysia*
[c]*Faculty of Civil Engineering & Earth Resources, Universiti Malaysia Pahang, Gambang, Kuantan 25000, Malaysia*
Tel. +60 95493006; Fax: +60 95492998; email: zularisam@ump.edu.my

Received 5 July 2012; Accepted 14 February 2013

ABSTRACT

The control of membrane biofouling in the presence of *Piper betle* extract (PBE) as a biofouling reducer (BFR) has been investigated. Response surface methodology (RSM) has been employed to mitigate extracellular polymeric substances (EPS), transmembrane pressure (TMP) rise-up control, and dye removal in membrane bioreactor. Factors investigated were: BFR dosage, air flow rate, and hydraulic retention time (HRT). Optimum conditions found to be BFR of 0.23 mg/mg mixed liquor suspended solids, HRT of 30.16 h, and air flow rate of 0.60 l/min, with predicted values as 28.28 mg/l of EPS, 24.16 kPa of TMP, and 95.65% dye removal, respectively. Validatory tests were carried out under the optimum conditions, which were closely agreed with the predicted values. These results depicted that RSM was one of the suitable methods to optimize the operating conditions to mitigate fouling in the presence of a BFR. PBE was verified to mitigate membrane biofouling via inhibiting autoinducers production. Furthermore, it was found that the addition of PBE decreased the amount of EPS in biocake; whereas the addition of autoinducer increased it. These findings revealed that PBE could be a novel agent to target quorum sensing, to mitigate membrane biofouling.

Keywords: Response surface methodology; Extracellular polymeric substances; Biofouling reducer; Quorum sensing

1. Introduction

The textile sector is the third largest foreign exchange earner in Malaysia after the palm oil and electronic industries [1]. Approximately 1,500 textile factories operate in Malaysia [2]. These textile facilities discharge a large amount of wastewater [3]. Wastewater dye affects water transparency, gas solubility, and can be toxic to aquatic organisms [4]. Moreover, most synthetic azo dyes are toxic and carcinogenic [5]. Textile wastewater is often treated with physio-chemical methods, but these methods are generally very expensive [6]. Moreover, the complex molecular structure of dyes makes them more resistant to degradation via physio-chemical methods.

Membrane bioreactors (MBRs) have appeared as a leading technology in wastewater treatment [7].

*Corresponding author.

However, MBRs are hindered by biofouling, which reduces membrane life, increases membrane cost, decreases permeate flux, and ultimately adds extra cost for membrane replacement. Accordingly, the mitigation of membrane biofouling is a major problem and a challenging task [8].

Biofouling is related to the occurrence of microbes, which can damage the membrane. Bacterial cells can enclose themselves in the structure of a matrix of extracellular polymeric substances (EPS) and form gel layers known as biofilm [9]. Moreover, biofilms are dynamic in nature, with structures that comprise a sequential process, in which colonizing bacteria move to the membrane surface, attach, and then produce a biofilm via a succession of steps [10]. EPS, which are produced by bacterial cells, are composed of a variety of components such as proteins, polysaccharides, and lipids [11]. EPS are known to be major biofouling causing substances in MBRs [12]. Indeed, increases in EPS have been shown to cause reductions in membrane flux in MBRs [13]. However, further research regarding EPS, their effects on biofouling, and EPS concentration mitigation is needed for batik wastewater to fully understand their role in MBR applications.

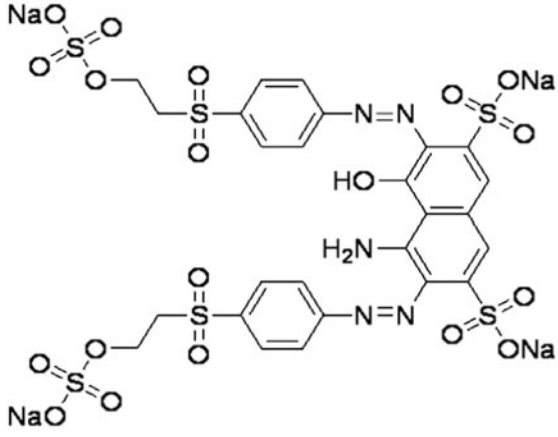
In conventional experiments, optimization is achieved by changing a single factor, while keeping others constant. This technique is costly and time consuming. Moreover, possible interaction effects between variables cannot be assessed, and it will lead to misleading conclusions.

Response surface methodology (RSM) is a type of technique for designing experiments and building models and determining optimum conditions for desirable responses [14]. This technique is applied to find the optimum operating conditions [15]. This technique can not only assess the interactions effects among confirmed operating variables, but also lessen the number of experiments to be carried out [16]. Recently, RSM has been used effectively to the optimization of operating conditions in wastewater treatment [17].

Reactive black 5 has been extensively used in textiles and printing industry for its fastness to light, perspiration, and repeated washing. Moreover, it cannot be easily disposed by conventional physical or chemical methods due to its stable chemical structure [8].

In this study, the control of membrane fouling in the presence of *Piper betle* extract (PBE) as a fouling reducer has been investigated. RSM has been employed to control EPS, transmembrane pressure (TMP) rise-up, and dye removal in MBR by investigating the single and interactive effects of three significant operating variables: PBE dosage, air flow rate and hydraulic retention time (HRT). The processes were designed in accordance with central composite design (CCD) and carried out in submerged MBR. Additionally, to verify whether the PBE addition could mitigate the membrane biofouling based on quorum sensing (QS) was investigated.

Table 1
The chemical structure and properties of reactive black 5

Chemical structure	
Molecular formula	$C_{26}H_{21}N_5Na_4O_{19}S_6$
Molecular weight	991.82
(nm)	597
Solubility	Soluble in water

2. Materials and methods

2.1. Dye and dye solution

Reactive black 5 (306452, Sigma-Aldrich) was purchased from Permula chemicals, SDN. BHD., Malaysia. Reactive black 5 stock solutions were prepared by dissolving (pH 6.5) 1.00 g of dye into 1 l of boiling distilled water. The chemical structure and properties of reactive black 5 are shown in Table 1.

2.2. Preparation of the activated sludge and biofouling reducer (BFR)

Sludge sample was collected in a sterilized polyethylene bottle from a storage tank of colored batik effluent (Natural Batik Village, Kuantan, Malaysia). Sludge collected was initially dried overnight at 40˚C and then sieved through 2 mm mesh sieve to remove large suspended material from it. In order to make sludge inoculum, 5 g of sludge was added to 100 ml distilled water, containing 1 g glucose, and the mixture was a shaken for 24 h on shaker (100 rpm) incubator at 30˚C before use.

P. betle L. leaves were obtained from Mentakab, Pahang, Malaysia. Fresh healthy leaves were washed with distilled water and air dried. Dried leaves were shredded into small pieces. 100 g of pieces were boiled in 1 l of deionized distilled water for many hours until the final volume was 100 ml. Further, the extract was centrifuged at 10,000 rpm to remove sediments. The supernatant was divided into 1 ml aliquots, in microfuge tubes. It was concentrated using a speed vacuum concentrator. The extracts were weighed into sterile micro-fuge vials and prepared into stocks of 20 mg/ml, using sterile distilled water as diluents, and sterilized by a 0.2 μm membrane filter. The PBE (pH 6.5) was completely soluble in water. The extracts were dissolved by sonicating the microfuge vials in a sonicator (DAIHAN) and stored at 4˚C until use.

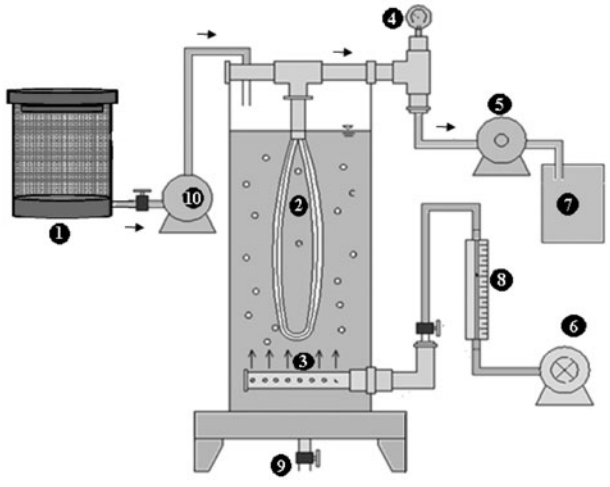


Fig. 1. The reactor setup: (1) feed tank, (2) hollow fiber membrane, (3) air splitter, (4) pressure gauge, (5) peristaltic pump, (6) air compressor, (7) effluent storage tank, (8) air flow meter, (9) drain valve, and (10) feed pump.

2.3. Submerged membrane bioreactor (SMBR) system and operation

Fig. 1 illustrates a laboratory scale ultrafiltration (UF) MBR system similar to the one used in another work [18] with slight modifications. All tests performed in this study were carried out in a SMBR. The bioreactor had a working volume of 2 l. A loop shaped polysulfone hollow fiber bundle with an effective area of 0.05 m^2 was inserted into the bioreactor. The specifications of the hollow fiber bundle are presented in Table 2. The reactor was seeded with activated sludge (10%) inoculum, and was operated for around 20 days for acclimatization. The synthetic dye wastewater, having COD concentration of 3,502 mg/l ± 010, was fed continuously to the bioreactor. The composition of synthetic dye wastewater was as follows; 503 mg/l glucose, 1,165 mg/l peptone, 834 mg/l yeast extract, 300 mg/l urea, 167 mg/l KH_2PO_4, 1,165 mg/l Nacl, 34 mg/l $MgSO_4.7H_2O$, and 100.5 mg/l reactive black 5 (dye) [8]. The membrane permeate was suctioned out through the hollow fiber membrane by a peristaltic pump and permeate was collected in a vessel. Other operating conditions are given in Table 3. The membrane fouling was monitored by the increase in TMP difference using a vacuum gauge meter. All experiments were conducted at 25–30˚C.

Table 2
Membrane and membrane bundle specifications

Membrane		Membrane bundle
Model	DL-F/K11-UF	
Material	Polysulfone	
Membrane type	Hollow fiber	
Pore size	0.03 μm	
Surface area	0.05 m^2	
Module		
Configuration	Loop-shaped hollow fiber	
Fiber outer diameter	600 μm	
Fiber internal diameter	300 μm	
Sampling point	Middle	

Table 3
Operating conditions used for reactor

Conditions	
Working volume (l)	2
TMP (kPa)	<33
Constant flux (l/m^2 h)	5.2
Air flow rate (l/min)	0.60–1.40
pH	6.5–7.5
OLR (mg COD/l/day)	2,333.33
HRT (h)	30–42
MLSS (mg/l)	4,451 (±200)

2.4. Experimental design and optimization

RSM based on CCD was applied to optimize the experimental conditions for reactive black 5 decolorization, EPS mitigation and TMP control. Three critical parameters: PBE dosage (x_1), HRT (x_2), and air flow rate (x_3) were selected as the independent variables based on the preliminary experiments, and EPS (y_1), TMP rise (y_2) and decolorization (y_3) were considered as the dependent variables (responses). Experimental range and levels of independent variables for reactive black 5 (dye) decolorization; EPS, and TMP were presented in Table 4.

Experimental conditions of CCD runs of Design-Expert® 8.0.7.1 (trial) and responses are presented in Table 5. In this study, the total number of experiments with three factors were 20 ($=2^k + 2k + 6$), where k is the number of factors. The first 4 columns show run numbers and experimental conditions of runs as arranged by CCD. Performance of the process was evaluated by analyzing the response of EPS, TMP, and dye removal. As there are only three levels for each parameter, the appropriate model is the quadratic Eq. (1).

$$y = b_o + \sum b_i x_i + \sum b_{ii} x_i^2 \sum b_{ij} x_i x_j \quad (1)$$

where y is the response variable, b is the regression coefficient of the model, x is the coded levels of the independent variables.

This model is preferred because a relatively few experimental combinations of the independent variables are adequate to estimate potentially complex response function. Analysis of variance (ANOVA) was executed to obtain the process factors and responses. The quality of the fit of model was expressed by determination coefficient R^2 and adjusted R^2 (R^2_{adj}), and statistical significance was evaluated by the F-values and P-values. Model terms were selected or rejected based on the probability value with 95% confidence level. Finally, three dimensional response surfaces were drawn in order to visualize the individual and the interactive effects of the independent variables. The desired goals were selected as maximum for dye removal, while minimum for EPS and TMP. The HRT and air flow rate were set as minimum, in order to reduce time and consumption of electrical energy. To test the validity of the predicted models, additional three runs were carried out under the optimum conditions obtained through RSM.

Table 4
Experimental range and levels of independent variables

Independent variable	Factor	Range and levels	
	x_i	−1	+1
PBE concentration (mg/mg MLSS)	x_1	0.20	0.40
HRT (h)	x_2	30.0	42.0
Airflow rate (l/min)	x_3	0.60	1.40

2.5. QS signal compound and testing of PBE for its QS inhibition efficacy

The QS signal compound; *N*-hexanoyl-DL-homoserine lactone (C6-HSL) [HHL] was procured from Sigma-Aldrich. Controlling the *N*-acyl homoserine lactones (AHL)-regulated gene expression gives a novel way to mitigate unwanted bacterial activity without killing bacteria. The PBE, was tested for its QS inhibition efficacy to mitigate membrane biofouling. MBR was operated for 4 days, and PBE optimum dosage 0.23 mg/mg mixed liquor suspended solids (MLSS) was added in the bioreactor. In the first step, role of PBE was carried out using specific concentration of PBE (0.23 mg/mg MLSS) and its effect on TMP was observed. In the last step, a study was carried out that how QS activity mitigates biofouling of membrane. We measured the amount of EPSs (mg/g biocake) under the various operating conditions.

2.6. Analytical

Measurements of reactive black 5 (dye) concentrations were carried out according to the Standard Methods for the Examination of Water and Wastewater [19]. The soluble EPS concentrations were analyzed by physical-chemical extraction method [20]. The soluble EPS was obtained as the supernatant of the centrifuged mixed liquor. Soluble EPSs were analyzed for proteins and polysaccharides, which are the dominant components in EPS and the sum of polysaccharides and proteins was taken as the total amount

Table 5
CCD with experimental and predicted results

Run no.	Factor variables			Experimental			Predicted		
	x_1 (mg/mg)	x_2 (h)	x_3 (l/min)	y_1 (mg/l)	y_2 (kPa)	y_3 (%)	y_1 (mg/l)	y_2 (kPa)	y_3 (%)
1	0.5	36	1.0	41.13	29	93.43	42.53	29	93.44
2	0.3	24	1.0	34.94	24	93.53	35.82	25	93.54
3	0.4	42	0.6	38.01	29	94.64	36.11	29	94.61
4	0.3	36	1.0	26.21	24	96.27	27.03	25	96.26
5	0.3	36	1.8	29.82	26	95.08	30.32	26	95.04
6	0.3	48	1.0	35.03	28	95.08	35.30	28	95.03
7	0.3	36	1.0	26.39	26	96.28	27.03	26	96.26
8	0.4	30	1.4	37.83	26	93.51	36.15	25	93.50
9	0.3	36	0.2	28.12	25	95.15	28.77	24	95.17
10	0.3	36	1.0	26.64	25	96.25	27.03	25	96.26
11	0.1	36	1.0	39.73	28	95.53	39.48	28	95.49
12	0.3	36	1.0	28.37	25	96.3	27.03	25	96.26
13	0.2	30	0.6	31.06	25	95.54	29.68	25	95.51
14	0.3	36	1.0	27.83	24	96.23	27.03	25	96.26
15	0.4	42	1.4	31.94	27	95.12	31.72	27	95.17
16	0.2	30	1.4	35.32	27	94.77	35.62	27	94.82
17	0.4	30	0.6	32.36	26	94.79	32.20	25	94.79
18	0.2	42	1.4	31.74	27	96.46	31.19	27	96.49
19	0.3	36	1.0	25.59	25	96.28	27.03	25	96.26
20	0.2	42	0.6	32.63	26	95.3	33.60	26	95.34

of EPS [21]. Protein concentration was determined by Lowry's method [22] and polysaccharides by the phenol-sulphuric acid method [23], using glucose as a standard.

The concentration of reactive black 5 (dye) was determined by measuring the absorbance at 590 nm with a spectrophotometer (U-1800, HITACHI). Decolorization rate of reactive black 5 was calculated as below:

$$\text{Decolorization } \% = \frac{A_0 - A}{A} \times 100 \quad (2)$$

where A_0 is influent dye concentration, and A is dye concentration in the effluent storage tank.

3. Results and discussion

3.1. Overall performance

Twenty CCD runs were executed to investigate the effect of independent parameters on responses. The results according to experimental conditions are presented in Table 5. The observed EPS, TMP, and dye varied in the ranges of 25.59–41 mg/l, 24–29 kPa, and 93.43–96.46%, respectively. Generally, first, an increase in PBE dose as a BFR in bioreactor resulted in decreased in EPS concentration and subsequently decreased the TMP. Secondly, increased air flow rate in the bioreactor system resulted in decrease of EPS and TMP, while increased removal of dye, finally longer HRT resulted in higher removal of the dye, decrease EPS concentration, and less elevation of TMP. As can be seen in Table 5, run 19 specifically gives the best results for EPS and TMP at 25.59 mg/l and 25 kPa, while more than 96% removal of dye was achieved in the same run at 0.30 mg/l of PBE concentration, 1 l/min AR, and 36 h HRT. For mitigation of EPS, four runs (Runs 4, 7, 10, and 19) showed the highest mitigation under different factor conditions. Run 2, 4, and 14 also give the least rise of TMP (24 kPa) under different factor conditions.

3.2. Fitting model and ANOVA

In order to study the effects of three independent variables on the EPS mitigation, TMP rise and dye removal were performed according to CCD and the results along with the experimental conditions were presented in Table 6. An approximate function of EPS, TMP, and dye removal based on experimental results was evaluated and given in Eqs. (3)–(5).

Table 6
ANOVA of the quadratic models for EPS, TMP, and Dye removal

Source	Sum of squares	Degree of freedom	Mean square	F value	Prob > F
EPS (mg/l)					
Model	409.72	8	51.21	29.670	<0.0001
x_1	9.287	1	9.287	5.3804	0.0406
x_2	0.267	1	0.267	0.1551	0.7012
x_3	2.379	1	2.379	1.3784	0.2652
x_1^2	306.92	1	306.92	177.809	<0.0001
x_2^2	114.35	1	114.35	66.247	<0.0001
x_3^2	9.943	1	9.943	5.760	0.0352
x_1x_3	1.970	1	1.970	1.141	0.3083
x_2x_3	34.81	1	34.819	20.172	0.0009
Residual	18.98	11	1.726		
Lack of fit	13.46	6	2.244	2.031	0.2269
Pure error	5.52	5	1.104		
$R^2 = 0.955$, $R^2_{adj} = 0.923$, $R^2_{pred} = 0.810$, adequate precision = 17.58					
TMP (kPa)					
Model	41.39	9	4.59	10.44	0.0005
x_1	1.56	1	1.56	3.54	0.0890
x_2	10.56	1	10.56	23.98	0.0006
x_3	0.56	1	0.56	1.27	0.2848
x_1^2	22.90	1	22.90	52.02	<0.0001
x_2^2	2.73	1	2.73	6.20	0.0320
x_3^2	1.05	1	1.05	2.38	0.1532
x_1x_2	1.12	1	1.12	2.55	0.1410
x_1x_3	3.12	1	3.12	7.09	0.0237
x_2x_3	1.12	1	1.12	2.55	0.1410
Residual	4.40	10	0.44		
Lack of fit	1.57	5	0.31	0.55	0.73
Pure error	2.83	5	0.56		
$R^2 = 0.903$, $R^2_{adj} = 0.8173$, $R^2_{pred} = 0.6376$, adequate precision = 10.42					
Dye removal (%)					
Model	18.01	8	2.25	1,325.62	<0.0001
x_1	4.21	1	4.21	2,479.52	<0.0001
x_2	2.25	1	2.25	1,328.71	<0.0001
x_3	0.01	1	0.018	11.12	0.0066
x_1^2	5.07	1	5.07	2,989.12	<0.0001
x_2^2	6.11	1	6.11	3,599.40	<0.0001
x_3^2	2.12	1	2.12	1,250.40	<0.0001
x_1x_3	0.17	1	0.17	104.18	<0.0001
x_2x_3	1.70	1	1.70	1,001.76	<0.0001
Residual	0.018	11	0.001		
Lack of fit	0.015	6	0.002	4.21	0.06
Pure error	0.003	5	0.0006		
$R^2 = 0.992$, $R^2_{adj} = 0.99$, $R^2_{pred} = 0.99$, adequate precision = 110.48					

$$y_1 = 27.03 + 0.76x_1 - 0.13 + 0.39x_3 + 3.49x_1^2 + 2.13x_2^2 + 0.63x_3^2 + 0.2x_1x_2 - 0.50x_1x_3 - 2.09x_2x_3 \quad (3)$$

$$y_2 = 24.91 + 0.31x_1 + 0.81x_2 + 0.19x_3 + 0.95x_1^2 + 0.33x_2^2 + 0.20x_3^2 + 0.38x_1x_2 - 0.62x_1x_3 - 0.37x_2x_3 \quad (4)$$

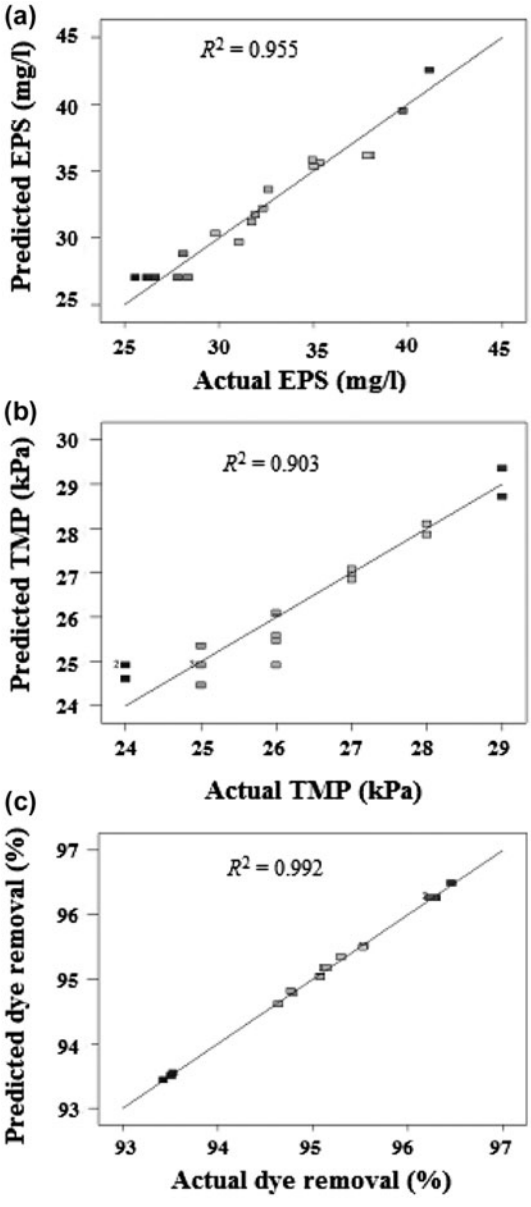


Fig. 2. Actual and predicted values of (a) EPS, (b) TMP, and (c) dye removal.

$$y_3 = 96.26 - 0.51x_1 + 0.38x_2 - 0.034x_3 - 0.45x_1^2 - 0.49x_2^2 - 0.29x_3^2 - 0.15x_1x_3 + 0.46x_2x_3 \quad (5)$$

In Eq. (3), y_1 is the EPS mitigation, in Eq. (4), y_2 is the TMP rise, and in Eq. (5), y_3 is the dye decolorization efficiency, respectively. While, x_1, x_2, and x_3 are corresponding coded variables of PBE dosage, HRT, and air flow rate, respectively.

ANOVA results of the quadradic models presented in Table 6, indicated that the model equations can adequately be used to describe the EPS mitigation, TMP rise control, and dye removal under a wide range of operating conditions. As can be seen, the model *F*-values for EPS, TMP, and dye removal were evaluated as 29.68, 10.45 and 1325.53, respectively. These values indicate the quadradic models are significant. An adequate level of precision, measures the signal to noise ratio and a ratio greater than 4 is desirable. In the obtained quadradic models of the EPS, TMP, and dye removal, the ratios of 17.58, 10.42, and 110.48 indicated an adequate signal for the models to be used to navigate the design space. For all models, $p > F$ values less than 0.05 indicate that the model terms are significant and hence these quadradic functions can be applied for successful prediction of future responses for EPS mitigation, TMP control, and dye removal. The obtained lack of fit *F*-values of 2.03, 0.55, and 4.22 indicate the insignificance for EPS, TMP, and dye removal, respectively. Fit of the model can be judged by correlations coefficients. The values of correlation coefficients of R^2 and R^2_{adj} were evaluated as 0.95 and 0.92 for EPS mitigation (Fig. 2(a)), as 0.90 and 0.81 for TMP control (Fig. 2(b)) and as 0.99 and 0.99 for dye removal (Fig. 2(c)). These regression values imply that the regression models fits to the experimental values well, and it can provide an excellent explanation of the relationships between the independent variable and the response.

3.3. Adequacy check of the model

Good adequacy can ensure that the approximating model provides an adequate approximation to the real system [24]. The diagnostic plots shown in Fig. 2(a–c) were used to estimate the adequacy of regression models. The actual and predicted EPS mitigation, TMP rise, and dye removal were shown in Fig. 2(a–c). The actual value is a measured value for a particular run, and the predicted value is evaluated from the model. In designed experiments, R^2 is a measure of the amount of reduction in variability of the response obtained by independent variables in the model. R^2_{adj} is preferred to be used to determine the fit of a

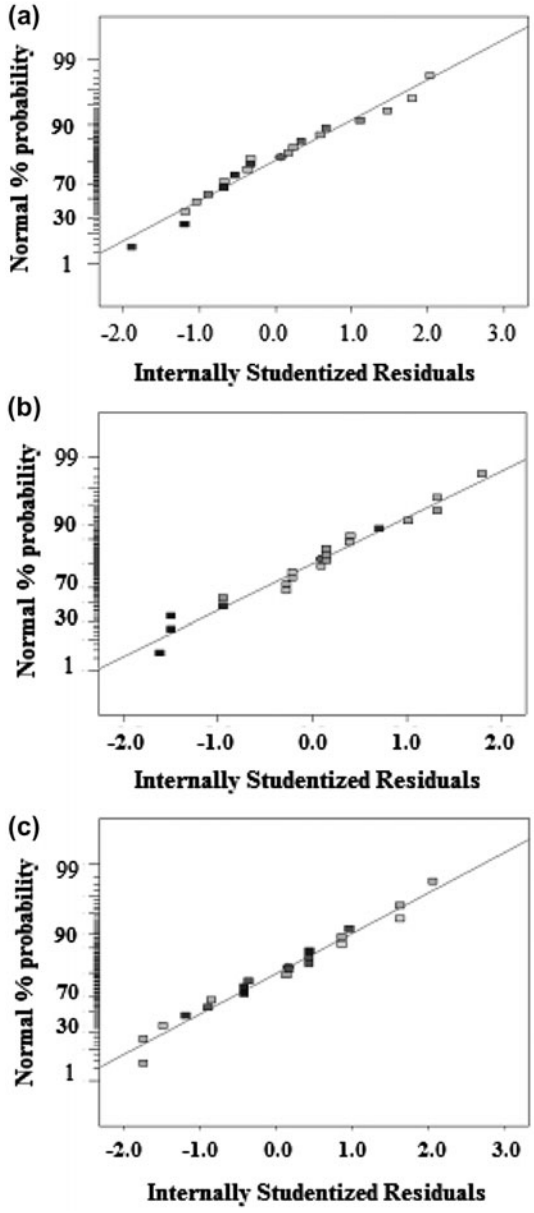


Fig. 3. The internally studentized residuals and normal % probability plot for (a) EPS, (b) TMP, (c) dye removal.

regression model, as it does not always increase when variables are added. The values of correlation coefficients of R^2 and R^2_{adj} were evaluated as 0.95 and 0.92 for EPS mitigation (Fig. 2(a)), as 0.90 and 0.81 for TMP control (Fig. 2(b)), and as 0.99 and 0.99 for dye removal (Fig. 2(c)), which shows good agreement between the predicted values and the experimental values, hence implies that the proposed model has adequate approximation to the actual value.

Residuals indicate how well the model satisfies the assumptions of ANOVA, whereas the internally studentized residuals measure the standard deviations separating the actual and predicted values [7]. The normal percent probability vs. studentized residual graphs for responses y_1, y_2, and y_3 yielded fairly straight lines (Fig. 3(a–c)), showing normal distribution of the data. The residual plots indicated a normal distribution, as no response transformation was needed and there was no apparent problem with normality in all cases.

3.4. Optimization conditions and response surface analysis

Optimization of simultaneous EPS mitigation, TMP control, and dye removal was conducted to determine the optimum factor conditions, applying the approximate models in Eqs. (3)–(5). The desired goal for each operational condition was preferred as minimum for HRT and air flow rate to reduce time and consumption of electrical energy. Through the optimization program (Design-Expert® 8.0.7.1 trial), the individual desirability was combined into a single number and then searched to maximize the model (Eqs. (3)–(5)) functions. The optimized conditions under specified goals were obtained to a level of desirability of 0.890 at 0.23 of PBE dosage (mg/mg MLSS), 0.60 of air flow rate (l/min), and 30.17 HRT (h). At these optimized conditions, 28.27 mg/l of EPS, 24 kPa TMP rise, and 95.65% dye removal were predicted using Eqs. (3)–(5), respectively.

The obtained Eq. (3) was used to visualize the effects of experimental parameters on EPS mitigation under optimized conditions, as depicted in Fig. 4(a–g). The interaction of air flow rate and BFR dosage at optimal HRT of 36 h is shown in Fig. 4(a). EPS concentration apparently increased with increasing air flow rate, while a different trend was found when PBE dosage was increased from 0.20–0.27 mg/mg MLSS, the concentration of soluble EPS decreased and having a minimum concentration (27.25 mg/l) at the BFR dosage of 0.27 mg/mg MLSS. The decrease in soluble EPS can be attributed to the flocculation process in which soluble foulants are entrapped [24]. The decrease in soluble EPS would have a positive effect on membrane permeability, because soluble EPS could provide a protective gel matrix for bacterial cells and can induce the blockage of membrane pores.

The HRT has a significant impact on mixed liquor as the change in HRT alters the organic loading rate (OLR). The shorter the HRT, the highest is the OLR. A change in HRT alters the biomass characteristics in the activated sludge system, which in turn changes the propensity of MBR fouling. Fig. 4(b) illustrates the response of EPS mitigation to the interaction of HRT with air flow rate at optimum PBE dosage of 0.3 mg/mg MLSS. The minimum EPS achieved at

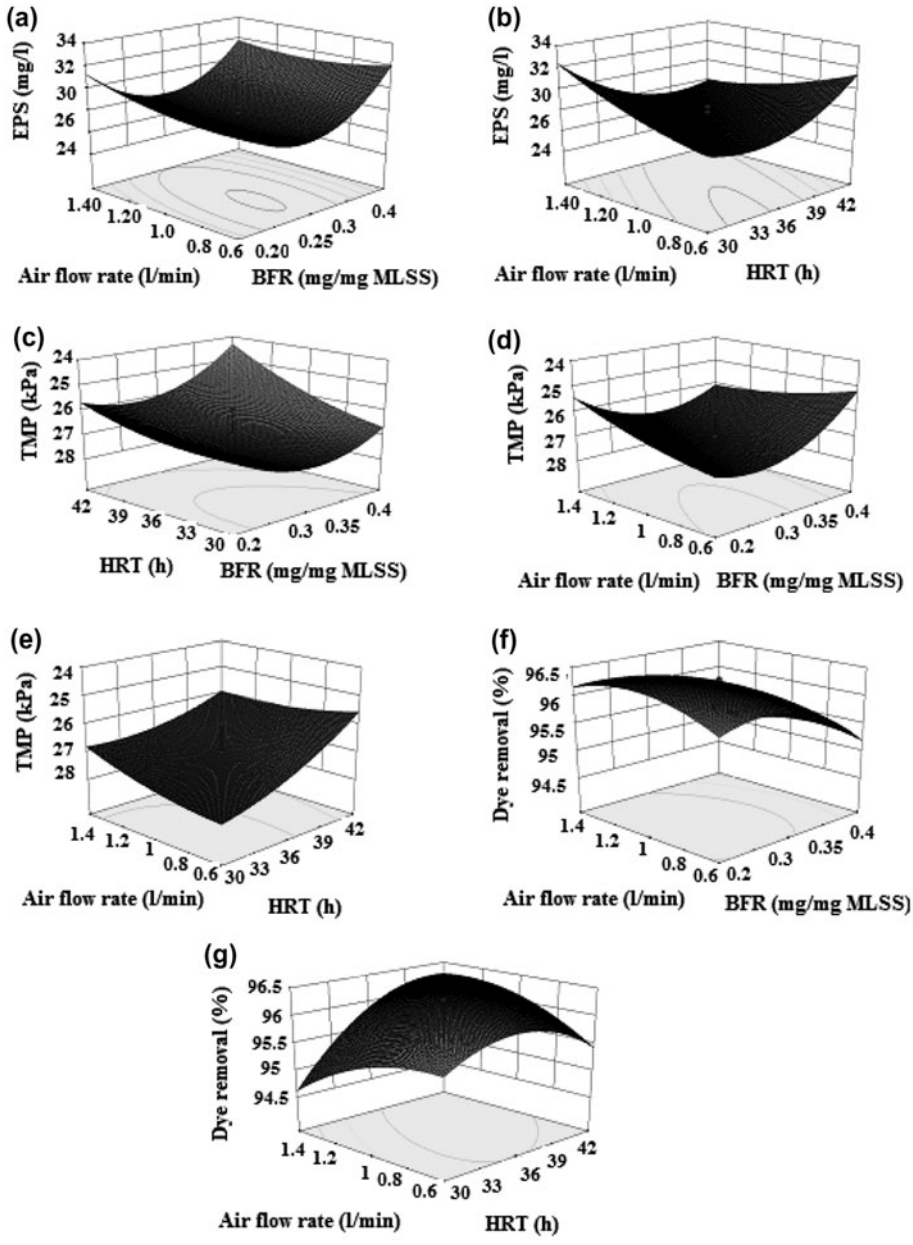


Fig. 4. Response surface for EPS (a)–(b), TMP (c)–(e), and for dye removal (f)–(g).

HRT of 33 h and air flow rate of 0.6 l/min with a concentration of 27.05 mg/l. However, the increase in HRT from 33 to 36 h showed an increase in EPS concentration (27.0531.22 mg/l). Fig. 4(b) also shows that with an increase of HRT from 33 to 44 h increased the EPS concentration. Previous literatures demonstrated that the overgrowth of biomass could result in much more release of EPS, and did great harm to membrane permeation [25]. Thus, it can be concluded that the increase of EPS concentration in MBR was mainly resulted from the over growth of bacteria [25]. It was also found that with increasing air flow rate, the EPS concentration was also increased, which might be due to the breakage of sludge flocs and higher production of EPS [26].

Fig. 4(c), shows TMP rise as it interacts with HRT and BFR dosage at optimal air flow rate of 1 l/min, BFR dosage and airflow rate at optimal HRT of 36 h (Fig. 4(d)) and the effects of HRT and air flow rate at optimal PBE dosage of 0.30 mg/l (Fig. 4(e)). Fig. 4(c) shows that with increasing PBE dosage from 0.2 to 0.3 mg/mg MLSS, the TMP rise was decreased, but when PBE dosage was further increased, the TMP started increasing. However, when HRT was

increased from 30 to 33 h, TMP rise decreased and further increase in HRT increased the TMP. Fig. 4(d) shows that minimum rise of TMP was obtained at 0.3 mg/l PBE dosage and 30 h HRT. With increasing PBE dosage from 0.2 to 0.25 mg/l, the TMP was decreased. The rate of TMP increase is a key factor in assessing membrane filterability in submerged MBR systems because it is directly associated to the extent of membrane fouling. Membrane fouling was thus reduced considerably by BFR dosage of 0.3 mg/l PBE dosage. Fig. 4(e) shows that the lowest increase in TMP was found at HRT of 30 h, while a further increase in HRT increased the TMP. Moreover, the same trend was found for air flow rate and the lowest TMP rise was found at flow rate of 0.60 l/min.

Fig. 4(f) shows dye removal with air flow rate and PBE dosage at HRT of 36 h. Initial increase in PBE dosage enhanced the dye removal (96%), while a further increase in PBE dosage from 0.30–0.40 mg/mg MLSS decreased the dye removal to 95%. At optimum concentration of PBE, the bacterial growth was controlled, but when the PBE concentration was further increased, it also reduced the dye removal, which might be due to the fact that the bacterial growth was inhibited. Moreover, increase in air flow rate from 0.6–1.0 l/min increased the% of dye removal. While, further increase in air flow rate decreased the % of dye removal. Fig. 4(g) shows the interaction between HRT and air flow rate at the optimal PBE dosage of 0.30 mg/mg MLSS. Increased in HRT from 30 to 36 h, increased the dye removal, while a further increase in HRT decreased the dye removal. The dye removal efficiency slightly increased as HRT increased from 30 to 36 h, due to the lower ORL. Moreover, highest dye removal was found at 0.6 l/min of air flow rate. The increase in air flow rate decreased the dye removal, which might be due to the fact that at higher air flow rate, breakage of microbial flocs occurred, which reduced the dye removal performance of MBR.

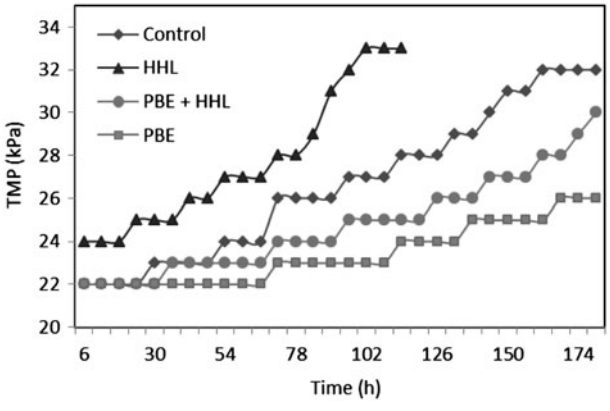


Fig. 5. TMP profiles: verification for biofouling mitigation by QS (PBE: 0.23 mg/mg MLSS, HHL: 1 mg/l).

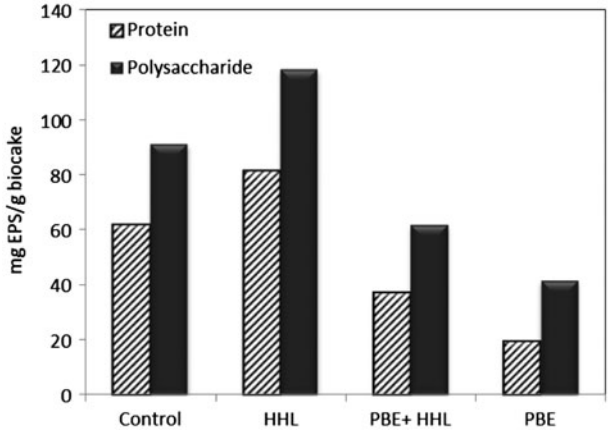


Fig. 6. Evidence for membrane biofouling mitigation by QS: quantitative analysis of EPSs in biocake in the MBR under various operating conditions (Reactor operation time: 4 days, PBE: 0.23 mg/mg MLSS, HHL: 1 mg/l).

3.5. Model validation and experimental confirmation

To test the predicted model, additional three runs were carried out under the optimum conditions obtained through RSM. In these confirmatory runs, simultaneous mitigation of EPS, TMP rise control and dye removal were found at 28.91 mg/l (EPS), 24.77 24.16 kPa (TMP), and 95.83% (dye removal), respectively, showing the accuracy of the model approach (Eqs. (3)–(5)), compared with the respective values of 28.28 mg/l (EPS), 24.16 kPa (TMP), and 95.65% dye removal obtained using the models.

3.6. Role of PBE to mitigate biofouling based on QS

It has been observed that the addition of PBE decreased the TMP rise in MBR (Fig. 5), which shows that PBE can mitigate biofouling. Therefore, further experiments were carried out to study the role of PBE in biofouling. In the first step, HHL, which was identified as major AHL by TLC (our own study), was added (1 mg/l) to the bioreactor. It was found that with the addition of HHL, the TMP increase was rapid compared to the control (Fig. 5). Though, PBE and HHL were added at once to the bioreactor, it was observed that PBE cannot mitigate the increase of TMP, which also verifies that the PBE cannot inactivate the AHL. Moreover, when PBE alone was added the increase of TMP became slower, which implies that PBE mitigated the production of AHL.

In the last step, a study was carried out that how QS activity mitigates biofouling of membrane. We

measured the amount of EPSs (mg/g biocake) under the various operating conditions (Fig. 6). It was found that with the addition of HHL; the increase of EPS (200.18 mg/g biocake) was rapid compared to the control. The addition of PBE in bioreactor decreased the EPS concentration (60.84 mg/g biocake), whereas the addition of an autoinducer in bioreactor increased (199.78 mg/g biocake) it. However, when PBE and HHL were added to bioreactor at the same time, it was observed that PBE cannot control the HHL activity, which further confirms that PBE controlled the production of AHLs. These results suggested that QS can be mitigated by controlling EPS concentration, but also shows a proof for the relationship between biofouling and QS.

4. Conclusion

The optimization of simultaneous EPS mitigation, TMP rise, and dye removal in MBR was studied using RSM based on CCD in the presence of a BFR. Regression models were proposed to describe the effects of independent variables and their interactions on EPS, TMP, and dye removal. ANOVA indicated the proposed regression models for EPS, TMP, and dye removal to be satisfactory fitted, with R^2 of 0.95, 0.90, and 0.99, respectively. The optimum conditions for EPS, TMP, and dye removal were PBE dosage of 0.23 mg/mg MLSS, air flow rate of 0.60 l/min, and HRT of 30.16 h, respectively, with predicted EPS, TMP, and dye removal of 28.28 mg/l, 24.16 kPa, and 95.65%, respectively. Moreover, confirmatory results were closely agreed with the predicted values. This study clearly showed that RSM based on CCD was one of the suitable methods to optimize the operating conditions to mitigate EPS, TMP, and dye removal in the presence of a BFR. PBE was verified to mitigate membrane biofouling via inhibiting AIs production. Furthermore, it was found that the addition of PBE decreased the amount of EPS in biocake; whereas the addition of HHL increased it. These findings revealed that PBE could be a novel agent to target AHLs for control of membrane biofouling. Further work can be carried out to isolate and purify the active compound of PBE to target the QS to mitigate membrane biofouling.

Acknowledgments

This research was supported (PGRS Grant No: GRS100308) by the Faculty of Chemical and Natural Resource Engineering, University Malaysia Pahang (UMP), Malaysia.

References

[1] Malaysian Textile Manufacturers Association, Statistic of Malaysian import and export (2008). Available from: http://www.fashion-asia.com/page.cfm?name=Statistic%20(Import%20and%20Export) (25 January 2010).
[2] A.L. Ahmad, W.A. Harris, S. Syafiie, B.S. Ooi, Removal dye from wastewater of textile industry using membrane technology, J. Teknol. 36(F) (2002) 31–44.
[3] N. Ali, A. Hameed, M.F. Siddiqui, P.B. Ghumro, S. Ahmed, Application of *Aspergillus niger* SA1 for the enhanced bioremoval of azo dyes in simulated textile effluent, Afr. J. Biotechnol. 8(16) (2009) 3839–3845.
[4] M. Marcucci, I. Ciabatti, A. Matteucci, G. Vernaglione, Membrane technologies applied to textile wastewater treatment, Ann. NY Acad. Sci. 984 (2003) 53–64.
[5] M.F. Siddiqui, S. Andleeb, N. Ali, P.B. Ghumro, S. Ahmed, Biotreatment of anthraquinone dye Drimarene blue K_2RL, Afr. J. Biotechnol. 4(1) (2010) 045–050.
[6] M.F. Siddiqui, S. Andleeb, N. Ali, P.B. Ghumro, S. Ahmed, Up-flow immobilized fungal column reactor for the treatment of anthraquinone dye Drimarene blue K_2RL, Afr. J. Biotechnol. 8(20) (2009) 5570–5577.
[7] T. Robinson, G. McMullan, R. Marchant, Remediation of dyes in textile effluent: A critical review on current treatment technologies with a proposed alternative, Bioresour. Technol. 77 (2001) 247–255.
[8] M.A. Yun, K.M. Yeon, J.S. Park, C.H. Lee, J. Chun, D.J. Lim, Characterization of biofilm structure and its effect on membrane permeability in MBR for dye wastewater treatment, Water Res. 40(1) (2006) 45–52.
[9] M.F. Siddiqui, M. Sakinah, A.F. Ismail, T. Matsuura, A.W. Zularisam, The anti-biofouling effect of *Piper betle* extract against *Pseudomonas aerugenosa* and bacterial consortium, Desalination 288 (2012) 24–30.
[10] M.J. Boorsma, S. Dost, S. Klinkhamer, J.C. Schippers, Monitoring and controlling biofouling in an integrated membrane system, Desalin. Water Treat. 31 (2012) 347–353.
[11] S. Comte, G. Guibaud, M. Baudu, Relations between extraction protocols for activated sludge extracellular polymeric substances (EPS) and EPS complexation properties part I. Comparison of the efficiency of eight EPS extraction methods, Enzyme Microb. Technol. 38 (2006) 237–245.
[12] A. Drews, C.H. Lee, M. Kraume, Membrane fouling—a review on the role of EPS, Desalination 200(1–3) (2006) 186–188.
[13] A. Dror-Ehre, A. Adin, H. Mamane, Control of membrane biofouling by silver nanoparticles using *Pseudomonas aeruginosa* as a model bacterium, Desalin. Water Treat. 48 (2012) 130–137.
[14] A.A.L. Zinatizadeh, A.R. Mohamed, A.Z. Abdullah, M.D. Mashitah, M. HasnainIsa, G.D. Najafpour, Process modeling and analysis of palm oil mill effluent treatment in an up-flow anaerobic sludge fixed film bioreactor using response surface methodology (RSM), Water Res. 40 (2006) 3193–3208.
[15] M. Amini, H. Younesi, N. Bahramifar, A.A.Z. Lorestani, F. Ghorbani, A. Daneshi, M. Sharifzadeh, Application of response surface methodology for optimization of lead biosorption in an aqueous solution by *Aspergillus niger*, J. Hazard. Mater. 154 (2008) 694–702.
[16] F. Karacan, U. Ozden, S. Karacan, Optimization of manufacturing conditions for activated carbon from Turkish lignite by chemical activation using response surface methodology, Appl. Therm. Eng. 27 (2007) 1212–1218.
[17] X. Li, R. Jia, P. Li, S. Ang, Response surface analysis for enzymatic decolorization of Congo red by manganese peroxidase, J. Mol. Catal. B: Enzym. 56 (2009) 1–6.

[18] A.W. Zularisam, A.F. Ismail, M.R. Salim, M. Sakinah, T. Matsuura, Application of coagulation–ultrafiltration hybrid process for drinking water treatment: Optimization of operating conditions using experimental design, Sep. Purif. Technol. 65 (2009) 193–210.
[19] APHA, Standard Methods for the Examination of Water and Wastewater, American Public Health Association, Washington, DC, 2005.
[20] T. Li, R. Bai, J. Liu, Distribution and composition of extracellular polymeric substances in membrane-aerated biofilm, J. Biotechnol. 135 (2008) 52–57.
[21] R. Bura, M. Cheung, B. Liao, J. Finlayson, B.C. Lee, I.G. Droppo, G.G. Leppard, S.N. Lis, Composition of extracellular polymeric substances in the activated sludge floc matrix, Water Sci. Technol. 37(4–5) (1998) 325–333.
[22] O.H. Lowry, N.J. Roserbrough, A.L. Farr, R.J. Randall, Protein measurement with the Folin phenol reagent, J. Biol. Chem. 193 (1951) 265–275.
[23] M. Dubois, K.A. Gilles, J.K. Hamilton, P.A. Rebers, F. Smith, Colorimetric method for determination of sugars and related substances, Anal. Chem. 28 (1956) 350–356.
[24] W.-N. Lee, I.-S. Chang, B.-K. Hwang, P.-K. Park, C.-H. Lee, X. Huang, Changes in biofilm architecture with addition of membrane fouling reducer in a membrane bioreactor, Process Biochem. 42 (2007) 655–661.
[25] F. Meng, H. Zhang, F. Yang, Y. Li, J. Xiao, X. Zhang, Effect of filamentous bacteria on membrane fouling in submerged membrane bioreactor, J. Membr. Sci. 272(1–2) (2006) 161–168.
[26] F.S. Fan, H.D. Zhou, Interrelated effects of aeration and mixed liquor fractions on membrane fouling for submerged membrane bioreactor process in wastewater treatment, Environ. Sci. Technol. 41 (2007) 2523–2528.