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Statistical optimization of pentachlorophenol biodegradation and electricity generation simultaneously in mediator – less air cathode microbial fuel cell

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Abstract

Microbial fuel cell (MFC) presents a novel method for simultaneous bioelectricity generation and pollutants treatment. In this study highly resistant and toxic pentachlorophenol (PCP) has been degraded as substrate in mediator – less air cathode MFC, to generate bioelectricity. Response surface methodology (RSM) based on central composite rotatable design (CCRD) was applied to determine the optimum condition for PCP degradation, power density and coulombic efficiency. Three operating variables, namely PCP – glucose concentration (mg/L), temperature (^{o}C) and pH, with a total of 15 individual experiments were conducted to optimize the combination effects of the variables. The predicted optimum conditions was 70-2500mg/L, 20 ^{o}C and 7.5 for PCP – glucose concentration (mg/L), temperature (^{o}C) and pH, respectively, resulting increasing PCP degradation from 58 to 73%, by rate 125%, power density from 7.84 to 23.08W/m2, by rate 294%, and coulombic efficiency from 39 to 49% by rate 126%. RSM based upon CCRD can be applied to correlate the statistical optimization results, with regression coefficients of 96.57, 94.95 and 96.4 for the PCP degradation, power density and coulombic efficiency, respectively. This proved that the RSM based on CCRD is efficiently applicable for statistical optimization of PCP biodegradation and electricity generation simultaneously in MFC. Dominant bacteria most similar to the anaerobic *Desulfobacterium aniline*, *Actinomycetes* and *Streptacidiphilus*, *Rhodococcus erythropolis*, *Amycolatopsis* and *Gordonia* were found on the anodic biofilm. These results demonstrate efficient degradation of PCP in MFC.

Keywords: Statistical optimization, pentachlorophenol degradation, microbial fuel cell.

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Introduction

Pentachlorophenol (PCP) is extensively used as herbicides, insecticides, fungicides, wood preservatives, resins, lubricants and intermediates of dyes. It is commonly found in ground waters, sediment and surface soils from dry areas near wood treatment plants, industrial wastewater effluents and treatment lagoons (Field and Sierra-Alvarez, 2008). PCP is acutely toxic to a variety of microorganisms and mammals, and is thought to inhibit oxidative phosphorylation (Shen et al., 2005). Besides, PCP could disrupt the proton gradient across membranes in cells (Escher et al., 1996; Ye et al., 2004), accumulates within the food chains and is considered to be mutagenic or at least comutagenic (Lu et al., 1997). It is of a significant risk to health of human beings (Dougherty, 1997). Therefore, it has been designated as a priority pollutant by the Environmental Protection Agencies in many countries (Chang et al., 1996). Microorganisms have been found in conventional biological processes that can degrade PCP (Field and Sierra-Alvarez, 2008; Karn et al., 2011; Li et al., 2010). Degradation of PCP has been shown to occur under anaerobic conditions (Mohn, 1992; Togna et al., 1995). The anaerobic degradation of PCP was investigated extensively in the literature (Chang et al., 1995; Duff et al., 1995; Hiroshi et al., 1996; Wilson et al., 1997; Piringer and Bhattacharya, 1999; Pieper et al., 2004). During anaerobic biodegradation, chlorines of PCP are removed from the aromatic ring via degradation (Mikesell and Boyd, 1985, 1986; Bryant et al., 1991) and the position of chlorine atom on the aromatic rings of PCP is an important factor that affects degradation. Remaining challenges are increasing PCP degradation rates, decreasing sludge generation, and lowering energy demands of the processes. Microbial fuel cells (MFCs) have recently been used for successful bioremediation of a number of chemicals (Huang et al., 2011a; Lovley and Nevin, 2011). An MFC is a device that uses microbes to convert the chemical energy stored in organic or inorganic compounds into electricity, providing a

low-cost and low-maintenance energy as well as a process that produces very little sludge (Huang et al., 2011a; Lovley and Nevin, 2011)

It is to be noted that the major part of the reported studies (Rao et al., 2000; Adour et al., 2008) did not implement mathematical and statistical methods for process optimization, and hence it only involves a separate optimization of each considered parameter with all others kept unchanged. Such empirical procedure needs several experimental runs, and hence it is time consuming, ignores interaction effects between the operating parameters and leads to low optimization efficiency. These limitations can be avoided by applying the response surface methodology that involves statistical design of experimentation in which all factors are varied together over a set of experimental runs. In fact, response surface methodology is a collection of mathematical and statistical useful techniques for developing, improving and optimizing processes, and it can be used to evaluate the relative significance of several affecting factors even in the presence of complex interactions (Khayet et al., 2011). This method involves various statistical and mathematical techniques based on fitting a polynomial equation and symmetric models to the experimental data in order to describe the behaviour of the independent variables (Bezerra et al., 2008). Among the second-order symmetric models, the most analytical process used is central composite rotatable design (CCRD), which has been widely applied in diverse scientific areas (Myers and Montgomery, 2002; Vargas et al., 2010; Auta and Hameed, 2011; Gan and Latiff, 2011; Prasad et al., 2011). It is supported by software, and it is an empirical modelization technique derived for the evaluation of the relationship a set of controlled experimental factors and observed results (Box and Wilson, 1951). As far as can be ascertained, the literature does not presently contain studies relating to investigating statistical optimization of PCP biodegradation and electricity generation simultaneously in mediator – less air cathode MFC involving central composite rotatable design (CCRD). Therefore, the present study proposed adapting the CCRD for optimizing PCP degradation in MFC. The relationship between responses (PCP degradation (%), power density (W/m^2) and coulombic efficiency (%),) and three independent factors (PCP – glucose concentration (mg/L), temperature (^oC) and pH) were investigated in this work.

Materials and methods

MFC construction

A single – chamber mediator – less MFC was constructed as described previously (Liu and Logan, 2004) with some modification. Briefly, the anode and cathode were placed in parallel on the opposite side of the chamber (total volume is 200 mL, working volume is 100 mL) with distance of 5cm. Non – wet proofed carbon cloth (type A,E – TEK, Somerset, NJ, USA, $4cm^2$) was used as anode. Wet – proofed (30%) carbon cloth (type B, E – TEK, Somerest, NJ, USA, $10cm^2$) was used as cathode pressed to proton exchange membrane (Nafion 117, Dupont CO., USA) on the water – facing side.

Inoculation and operation

To enrich electrochemically active bacteria during reactor startup, direct inoculation using domestic wastewaters has been investigated (Huang et al., 2011a, b; Liu et al., 2011). It was shown that domestic wastewater was a good inoculum for 1,2-dichloroethane removal (Pham et al., 2009), and therefore it was used here for inoculation. Prior to use, wastewater was sparged with N_2 gas for 15 min. Wastewater was initially combined with an equivalent volume of nutrient solution which contained (per L) NH_4HCO_3, 0.386g; $KHCO_3$, 0.149g; $NaH_2PO_4.2H_2O$, 3.31 g; $Na_2HPO_4.12H_2O$, 10.31 g; $MgSO_4.7H_2O$, 0.036 g; vitamins 12.5 mL/L and minerals 12.5 mL/L (pH 7.0, conductivity 6.5 mS/cm) (Lovley and Phillips, 1988). The reactor was operated in fed-batch mode. The pH was adjusted by adding NaOH or HCl. The temperature was controlled in an incubator (LAB – LINE ® AMBI – USA). The net volume of the anolyte was 100 mL for each experiment. Immediately after adding the fuel and inoculum, MFC was hooked up to a data acquisition system to start monitoring the voltage generation (150Ω).

Monitoring and calculation

Samples were withdrawn from the reactors using a syringe. The used solutions for PCP analysis were filtered through 0.22 µm pore diameter membrane filters and then analyzed using a high performance liquid chromatograph (HPLC Agilent 1100) equipped with a C_{18} capillary column (4.6 mm in diameter and 250 mm in length, ODS-2 Hypersil, Thermo). The mobile phase was trifluoroacetic acid in ultrapure water (pH = 2.8) and methanol (20:80 v/v). Standards were prepared for the following chlorophenols based on their presence in previous studies (Field and Sierra-Alvarez, 2008): 2,3,4,5-tetrachlorophenol (2,3,4,5-TeCP) (Supelco), 2,3,5,6-tetrachloro-1,4-hydroquinone (2,3,5,6-TeCHQ) (Chem Service), 2,3,6-trichloro-1,4-hydroquinone (2,3,6-TCHQ) (Chem Service), 2,6-dichloro-1,4-hydroquinone (2,6-DCHQ) (Aldrich), 3,4,5-trichlorophenol (3,4,5-TCP) (Supelco) and 3,5-dichlorophenol (3,5-DCP) (Supelco). PCP metabolites were confirmed by an APCI ($^-$) ion trap mass spectrometer coupled with LC (Agilent HPLC-MS/MS 6410). Electro-spray ionization was operated in a negative mode, with the scan mass range set from 70 to 350. Cl^- concentrations were analyzed by ion chromatography (ICS2500, DIONEX, USA) using an AS11-HC column and conductivity detector.

Voltage was measured after the MFC has reached the steady state by a digital multimeter (Sanwa CD800a, Japan) was connected to a personal computer. Data was automatically recorded every second via Picolog software (Pico Technology Limited). The corresponding current was based on equation $I=E/R_{ext}$, where : I is current (mA) , E is voltage (mV) and R_{ext} is external resistance. The power (P) was obtained by $P=IE$. The current density and the power density have been normalized based on the projected surface area of the anode via equations $I_{An}= I/A_{An}$, where I_{An} is current density and A_{An} is the surface area of anode, $P_{An}=E^2/A_{An}R_{ext}$, where P_{An} is power density. The polarization curve was obtained at different external resistance (50 - 1000Ω). Internal resistance was derived from the polarization curve as the slope. Coulombic efficiency (CE) was

derived from the equations $C_p=It$, $C_{max}=FfS_{COD}V_{An}$, and $CE=Cp/C_{max}$, where C_p is the coulombs of energy produced , t is the time of stable voltage output, C_{max} is the theoretical maximum coulombs , F is Faraday's constant (96.485 C/mol of electrons), f is a factor of 1mol electrons/8g COD, S_{COD} is substrate concentration g COD/l, and V_{An} is a net volume of anolyte (mL). COD was determined by standard method (Wei, 2002). All the experiments were replicated twice.

Bacterial community was analyzed using a polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE) technology. Total genomic DNA was firstly extracted using a PowerSoil DNA Isolation Kit (BioTeke Co. Ltd.) according to the manufacturer's instructions. The extracted DNA was then amplified using the universal primers 341f (50'-CCT ACG GGA GGC AGC AG-30') and 518r (50'-ATT ACC GCG GCT GCT GG-30'), and the products were amplified again with the primer set 341f, containing a GC clamp (50'-CGCCCGCCGCGCCCCGCGCCCGTCCCGCCGCCCCCGCC CG-30') (TaKaRa Co. Ltd., Japan). PCR (Applied Biosystems, Singapore) amplification was performed as follows: 4 min of pre-denaturation at 94°C, followed by denaturation at 94°C for 30s, 52°C for 30s and 72 °C for 1 min, and 30 cycles with a final extension at 72°C for 5 min. The PCR products (30 µL) were separated using 8% (wt/vol) polyacrylamide gels with a denaturant gradient between 30% and 70%. The Dcode Universal Mutation Detection System (Bio-Rad) was used for DGGE, which was first run in 1.0 × Tri–sacetate–EDTA buffer at 200 V for 30 min and subsequently at 100 V for 8 h (60 °C). After electrophoresis, the gels were stained using GoldView II (Solarbio, Shanghai) for 40 min and de-stained in 1.0 × Tri–acetate–EDTA buffer (pH 8.0) before the DNA bands were observed with a Gel-Doc image analyzer (Bio-Rad Laboratories). Bands of interest were excised from the gel and the PCR amplified products were purified using a DNA Purification Kit and then sequenced (Beijing Sunbiotech Co. Ltd.). The sequences were subjected to Basic Local Alignment Search Tool (BLAST). Sequences with similarities P97% were selected for phylogenetic analyses, which were performed using software packages of MEGA4 and Clustalx (1.81)

Experimental design

Central composite rotatable design (CCRD) was used as design of experiment as it provides much information with less number of experiments (Myers and Montgomery, 2002). In order to obtain required data, series of 15 experiments, including eight factorial points, six axial points and one central points was done based on three variables PCP – glucose concentration (mg/L), temperature (°C) and pH, derived from Equation (1) (Gan and Latiff, 2011).

$$N=2^n+2n+n_c=2^1+2(3)+7=15 \quad (1)$$

Before designing this experiment, suitable values for the three variables mentioned above, selected based on the preliminary study. CCRD with a full factorial was developed using STATISTICA software (Version 5.5, Stat-Soft Inc., USA). Each factor is varied over five levels: the high level (+), the low level (-), the centre points (coded level 0) and two outer points the very high (++) and the very low (--) (table 1) (Tan et al., 2008; Ghani et al., 2010; Ghani et al., 2011). The data, which has been obtained by design CCRD, are then used in the optimization. In this study, the response variables measured were PCP degradation (%), power density (W/m^2) and coulombic efficiency (%). The optimum condition for three variables, PCP – glucose concentration (A), temperature (B) and pH (C) were obtained using data from the statistical analysis. STATISTICA software searches for a combination of factors that simultaneously satisfy the requirements placed on each of the response and factors (Ghani et al., 2010).

Results and discussion

Start-up and control experiment

The MFC has been operated at conditions , initial PCP-glucose concentration 50-1500mg/L , temperature 25°C , pH 7.0. After 24 h following the inoculation of the anode in the MFC, voltage , power density and PCP degradation were increased slightly to 0.8 V , 1.1 W/m^2 and 0.2 % , respectively. Subsequently the voltage and power density recorded maximum values of 2.1 V and 7.8 W/m^2 , respectively , after 192 h of the operation (Fig. 1). At the experiment end (264 h) PCP degradation and coulombic efficiency were 58 and 39 % , respectively.

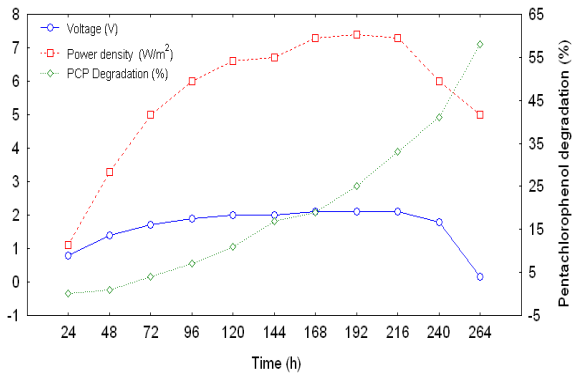

Fig 1. Time course of PCP degradation, Voltage and power density, which were produced on the control experiment.

Statistical analysis and optimization

The relationship between responses (PCP degradation, power density and coulombic efficiency) and three independent factors (PCP – glucose concentration, temperature and pH) were investigated in this study. Designed variables suggested by the software are shown in Table 1, while the experimental and predicted results at each point obtained are shown in Table 2. The experimental sequence was randomized in order to minimize the effects of the uncontrolled factors (Halim et al., 2009). For the model fitted, the software generated model coefficients, R^2-values, F-values, and significant probabilities, and from these values the significance of each experimental variable can be justified.

Table 1: Central composite design of independent variables PCP – glucose concentration (A), temperature (B) and pH (C) for process optimization.

Trial	Coded and actual levels		
	A (mg/L)(PCP-G)	B (^oC)	C
1	- (40-1000)	- (20)	- (6.5)
2	+ (70-2500)	- (20)	- (6.5)
3	- (40-1000)	+ (35)	- (6.5)
4	+ (70-2500)	+ (35)	- (6.5)
5	- (40-1000)	- (20)	+ (7.5)
6	+ (70-2500)	- (20)	+ (7.5)
7	- (40-1000)	+ (35)	+ (7.5)
8	+ (70-2500)	+ (35)	+ (7.5)
9	0 (50-1500)	0 (25)	- - (6.0)
10	0 (50-1500)	0 (25)	+ + (8.0)
11	0 (50-1500)	- - (15)	0 (7.0)
12	0 (50-1500)	++ (45)	0 (7.0)
13	- - (30-500)	0 (25)	0 (7.0)
14	++ (100-3000)	0 (25)	0 (7.0)
15	0 (50-1500)	0 (25)	0 (7.0)

Table 2: Experimental and predicted results for PCP degradation (PCP-D)(%), power density (P-D)(W/m^2) and coulombic efficiency (C-E)(%).

Trial	Experimental			Predicted		
	PCP-D	P-D	C-E	PCP-D	P-D	C-E
1	31	3.12	21	40	6.0	26
2	69	9.43	42	77	16.4	47
3	15	2.12	18	23	4.6	24
4	47	7.21	29	56	11.4	34
5	68	15.66	37	57	12.6	32
6	73	23.08	49	63	21.7	43
7	44	6.60	31	34	0.8	26
8	49	8.11	32	38	6.4	27
9	69	17.04	43	47	7.7	29
10	22	1.46	17	45	9.0	28
11	62	19.36	41	65	16.6	41
12	29	1.67	27	29	2.6	25
13	38	2.39	30	38	4.9	28
14	70	22.70	45	71	18.3	45
15 con	58	7.84	39	57	7.6	39

Table 3: ANOVA for the regression model and respective model term for PCP degradation.

Source	Sum of squares	Degree of freedom	Mean of square	*F*-value	Prob > *F*	Remarks
Model	1521.11	9	169.01	120.93	<0.0001	Significant
A	1191.65	1	1191.65	852.61	<0.0001	Significant
B	25.96	1	25.96	18.57	0.0020	Significant
C	20.62	1	20.62	14.75	0.0040	Significant
A^2	268.48	1	268.48	192.09	<0.0001	Significant
B^2	12.37	1	12.37	8.85	0.0156	Significant
C^2	0.76	1	0.76	0.54	0.4799	
AB	0.46	1	0.46	0.33	0.5799	
AC	2.08	1	2.08	1.49	0.2534	
BC	7.40	1	7.40	5.29	0.0470	Significant
Residual	12.58	9	1.40			
Lack of fit	9.68	5	1.94	2.67	0.1816	Not significant
Pure error	2.90	4	0.73			
Cor total	1543.23	19				

Table 4: ANOVA for the regression model and respective model term for power density

Source	Sum of squares	Degree of freedom	Mean of square	*F*-value	Prob>*F*	Remarks
Model	198.20	9	22.02	28.16	<0.0001	Significant
A	120.62	1	120.62	154.25	<0.0001	Significant
B	4.14	1	4.14	5.29	0.0470	Significant
C	3.06	1	3.06	3.091	0.0793	
A^2	63.56	1	63.56	81.29	<0.0001	Significant
B^2	2.15	1	2.15	2.75	0.1318	
C^2	1.17	1	1.17	1.50	0.2516	
AB	0.41	1	0.41	0.52	0.4900	
AC	1.28	1	1.28	1.64	0.2327	
BC	0.84	1	0.84	1.08	0.3257	
Residual	7.04	9	0.78			
Lack of fit	6.21	5	1.24	6.02	0.0532	Non significant
Pure error	0.83	4	0.21			
Cor total	206.82	19				

Table 5: ANOVA for the regression model and respective model term for coulombic efficiency.

Source	Sum of squares	Degree of freedom	Mean of square	*F*-value	Prob>*F*	Remarks
Model	2672.77	9	296.97	26.76	<0.0001	Significant
A	1896.26	1	1896.26	170.90	<0.0001	Significant
B	39.10	1	39.10	3.52	0.0932	
C	16.77	1	16.77	1.51	0.2501	
A^2	630.34	1	630.34	56.81	<0.0001	Significant
B^2	43.72	1	43.72	3.94	0.0784	
C^2	0.79	1	0.79	0.071	0.7959	
AB	17.55	1	17.55	1.58	0.2401	
AC	35.15	1	35.15	3.17	0.1088	
BC	6.11	1	6.11	0.55	0.4771	
Residual	99.86	9	11.10			
Lack of fit	88.24	5	17.65	6.07	0.0526	Not significant
Pure error	11.63	4	2.91			
Cor total	2772.64	19				

Statistical

In this study, the response and variables were fitted to each other by multiple regressions. Regression analysis is the general approach to fit the empirical model with the collected response variable data (DC, 2001). The coefficients of the full regression model equation and their statistical significance were determined and evaluated using STATISTICA 5.5 software from State-Soft Inc. The final model in terms of coded value is given in Equations (2) - (4) for PCP degradation, power density and coulombic efficiency.

$$Y_{PCP\text{-}D(\%)} = 40.85 - 9.34A - 1.38B - 1.23C + 4.32A^2 + 0{:}93B^2 + 0.23C^2 + 0.24AB + 0.51AC + 0.96BC \quad (2)$$

$$Y_{P.D(w/m^2)} = 28.42 + 2.97A + 0.55B - 0.47C - 2.10A^2 - 0.39B^2 + 0.29C^2 + 0.23AB \; 0.40AC - 0.32BC \quad (3)$$

$$Y_{C.E(\%)} = 39.63 - 11.78A - 1.69B - 1.11C + 6.62A^2 + 1.74B^2 - 0.23C^2 - 1.48AB - 2.10AC + 0.87BC \quad (4)$$

where Y is the response, and A, B and C are the coded terms for the three variables that has been selected, i.e. PCP – glucose concentration (A), temperature (B) and pH (C). Positive sign in front of each term represents synergistic effect, while antagonistic effect represented by negative sign. Analysis of Variance (ANOVA) was then used to assess the goodness of fit. The significant quadratic models and the corresponding significant model term for all responses are tabulated in Tables 3-5 for PCP degradation, power density and coulombic efficiency.

From Table 3, the model F-value of 120.93 implies that the model is significant. Interestingly, all of the variables PCP – glucose concentration (A), temperature (B) and pH (C) showed significant effects on the PCP degradation due to the high F-values of 852.61, 18.57, and 14.75, respectively. The quadratic term of PCP – glucose concentration (A2) and temperature (B2) have also significant effects with F-value of 192.09 and 8.85, respectively. The quadratic term of pH (C2), on the other hand, does not have effect on the PCP degradation significantly. Fig. 2 A, B and C showed the relationships between the variables to the PCP degradation.

From Table 4, the model F-value of 28.16 implies that the model is significant. It was also observed that the linear term of PCP – glucose concentration (A) has a large significant effect on the power density due to the high F-value of 154.25. The quadratic term of PCP – glucose concentration (A^2) has also a significant with F-value of 81.29. Temperature (B) also gives a significant effect to the power density with F-value of 5.29. However, pH (C) seems does not have any significant effect on the power density. The interaction between PCP – glucose concentration (AB) and pH (AC) also does not affect the power density significantly. The relationships between the variables are also shown in Fig. 3 A, B and C.

F-value of 26.76 shown in Table 5 implies that the model is significant for the response of coulombic efficiency. It was also observed that the linear term of PCP – glucose concentration (A) has a large significant effect on the coulombic efficiency due to the high F-value of 170.90. The quadratic term of PCP – glucose concentration (A^2) has also a significant effect with F-value of 56.81. However, temperature (B) and pH (C) do not affect the coulombic efficiency significantly. The interactions between the variables (AB, AC and BC) also do not affect the coulombic efficiency significantly.

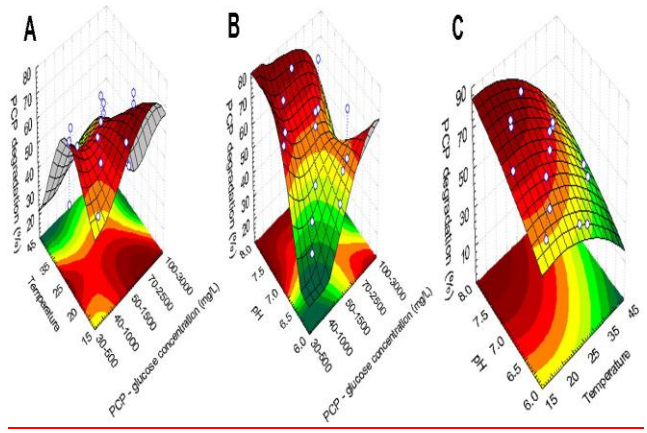

Fig. 2. Countour plot of PCP degradation: Effect of PCP-glucose concentration and temperature (A), effect of PCP-glucose concentration and pH (B) and effect of temperature and pH (C).

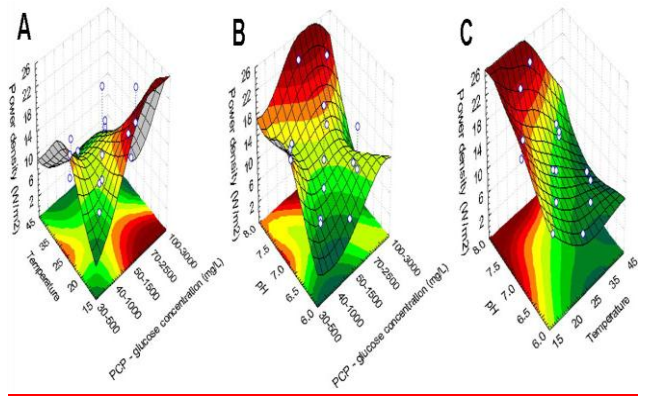

Fig. 3. Countour plot of power density: Effect of PCP-glucose concentration and temperature (A), effect of PCP-glucose concentration and pH (B) and effect of temperature and pH (C).

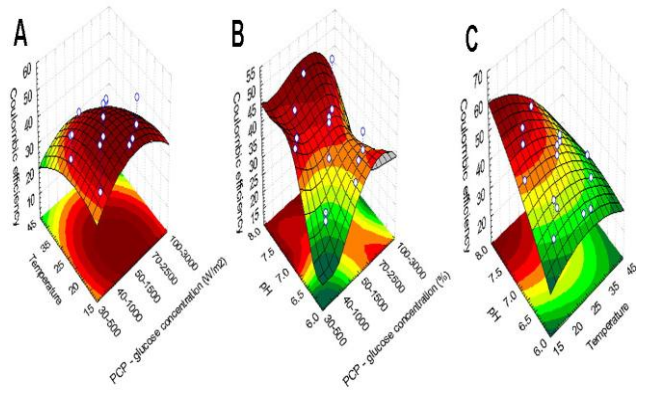

Fig. 4. Countour plot of coulombic efficiency: Effect of PCP-glucose concentration and temperature (A), effect of PCP-glucose concentration and pH (B) and effect of temperature and pH (C).

The relationships between the variables are shown in Fig. 4 A, B and C. Among the variables, PCP – glucose concentration (A) plays a major effect to all responses investigated in this study. By increasing the concentrations to 70-2500 mg/L , the responses have recorded highest values. These results may because of increasing population of microbial consortium in the anolyte. Temperature and pH were slightly modified to 20°C and 7.5 , without any significant effect. In order to test the fit of the model, the regression equation and the determination coefficient (R^2) were evaluated. For the response of PCP degradation, the value of determination coefficient (R^2 = 0.9657) indicates that the sample variation of 96.57% for PCP degradation is attributed to the independent variables, and only 3.43% of the total variation could not be explained by the model. The value of adjusted determination coefficient (Adj R^2 = 0.9314) is also very high to advocate for a high significance of the model. Meanwhile for the response of power density, the value of determination coefficient (R^2 = 0.9495) indicates that the sample variation of 94.95% for power density is attributed to the independent variables, and only 5.05% of the total variation could not be explained by the model. The value of adjusted determination coefficient (Adj R^2 = 0.8990) is also very high to advocate for a high significance of the model. On the other hand, the response of coulombic efficiency, the value of determination coefficient (R2 = 0.9640), indicates that the sample variation of 96.4% for coulombic efficiency is attributed to the independent variables, and only 3.6% of the total variation could not be explained by the model. The value of adjusted determination coefficient (Adj R^2 = 0.9280) is also very high to advocate for a high significance of the model.

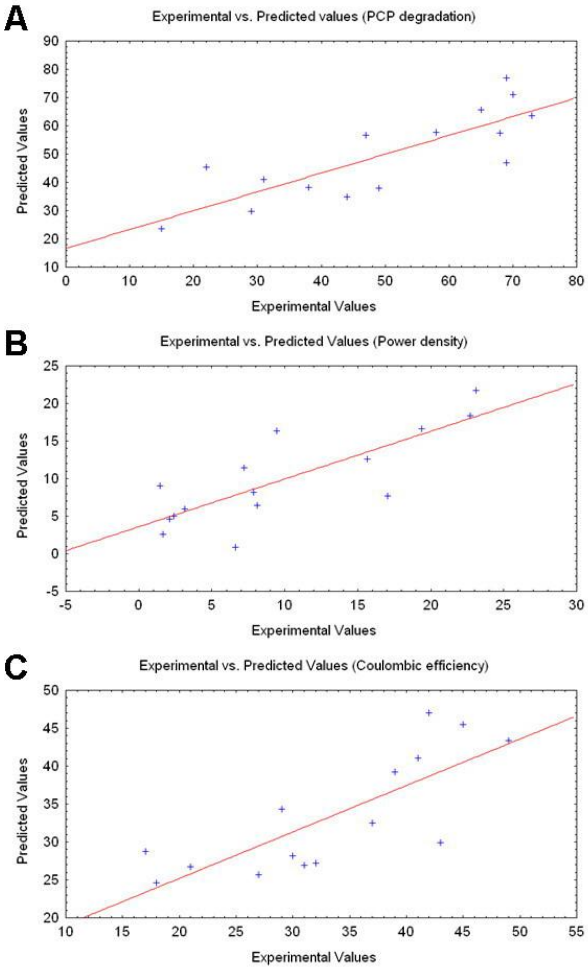

Fig. 5. Relationship between predicted and actual values of A) PCP degradation , B) power density, C) and coulombic efficiency.

The correlation between experimental values and predicted values of PCP degradation, power density and coulombic efficiency are shown in Fig. 5 A, B, and C, respectively. A

higher value of the correlation coefficient for all responses justifies an excellent correlation between the independent variables (Ghani et al., 2010; Ghani et al., 2010).

The optimized new levels of the variables (PCP – glucose concentration , temperature and pH) obtained by CCRD were 70-2500 mg/L , 20^{o}C and 7.5 , respectively, where values of PCP degradation, power density and coulombic efficiency recorded highest levels of 73 % , 23.08 W/m^2 and 49 % , respectively.

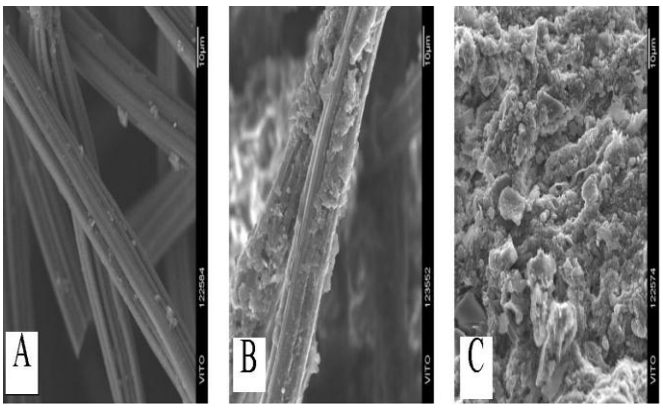

Fig. 6. SEM image of carbon cloth (Anode). A) Showed that the anode is clear of bacterial colonization before the experiments. B) Moderate growth of a biofilm has formed on the anode after the control experiment, and C) Heavy growth of bacteria is noted on the anode after statistical optimization experiments.

Bacterial morphologies and community

Fig. 6A showed that the anode is clear of bacterial colonization before the experiments. Then moderate growth of biofilm has been formed on the anode after the control experiment (Fig. 6B). Heavy growth of bacteria is noted on the anode after statistical optimization experiments (Fig. 6C) , which could explain the good system of performance after using CCDR. Biofilms on the anodes, which have been acclimated for 3, 2 and 1 d, were sampled for analyzing bacterial communities by DGGE, showing both common and different prominent bands (Fig. 7). Bands of C-3-5 (3 d) and C-1-1 (1 d) shared a phylogenetic relation to *Desulfobacterium aniline* and *Fusibacter*, although the C-1-1 became faint, demonstrating the gradual evolution of bacterialcommunities (Fig. 7). *Desulfobacterium aniline* can anaerobically mineralize multiple aromatic compounds including aniline, phenol, benzoate, 2-hydroxybenzoate, 4-hydroxybenzoate, 4-hydroxyphenylacetate, 2-aminobenzoate, 2-fluorophenol and 2-fluorobenzoate (Field and Sierra-Alvarez, 2008) whereas the mesophilic *Fusibacter* can efficiently dechlorinate tetrachloroethene (Lee et al., 2011). Based on the assumption that members that are closely related phylogenetically share similar metabolic capabilities, bacteria indicated to be present in bands C-3-5 and C-1-1 may thus mesophilically degrade aromatic intermediates from PCP degradation and explain the efficient system performance at a moderate temperature of 20 ^{o}C.

Similarly, sequences of C-3-2 were closely related to *Amycolatopsis* and *Gordonia*, which have been shown to degrade cholesterol and carbazole (Santos et al., 2006; Drzyzga et al., 2009). Bands C-2-1 (2 d) and C-3-1 shared the same sequences with the chlorophenols-degrading bacterium of *Rhodococcus erythropolis* (Goswami et al., 2002) and the diverse mono-chlorophenols degrading bacteria of *Actinomycetes and Streptacidiphilus* (Farrell and Quilty, 1999). Bands C-2-1, C-3-1 and C-3-2 indicated the presence of bacteria most similar to the *Actinomycetes* of *Amycolatopsis* sp. Bands of C-2-2, C-2-4, C-3-3 and C-3-4 formed several distinct clusters distant from the cluster of the uncultured *Nitrospira* sp., which are known to exist in environments contaminated with recalcitrant wastes such as pharmaceuticals (Kraigher and Mandic-Mulec, 2011).

Anodic PCP degradation is dependent on the activity of degrading microbes, which are phylogenetically diverse and belong to more than 100 species (Field and Sierra-Alvarez, 2008). While a variety of microorganisms were found on the biofilms on anodes which have been acclimated, many of which had a high similarity to known chlorophenol degrading bacteria. The reason for the presence of many other bacteria was unclear (Fig. 7). In addition, the electrotrophic activities and their role in chemolithotrophic degradation processes remain unknown. Current knowledge of bacterial communities contributing to anodic processes is limited to denitrifying (Wrighton et al., 2010), perchlorate (Butler et al., 2010) and oxygen reduction (Rabaey et al., 2008). Further investigation of the electrotrophic activities of bacteria with simultaneous PCP reduction with pure cultures is still needed.

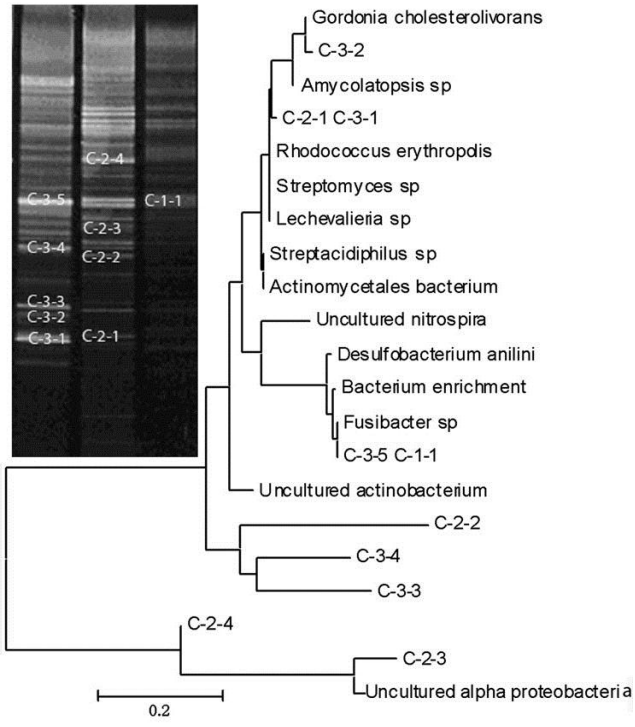

Fig. 7. Neighbor-joining tree based on 16S rRNA gene sequences derived from the DGGE band using Clustal X 2.0: anodic bacterial community profiles revealed by DGGE (from left to right: biofilms on the anodes acclimated for 3, 2 and 1 d, respectively. Bands C-3-1–C-3-5, C-2-1–C-2-4 and C-1-1 represented selected DGGE bands that were excised and sequenced).

Conclusions

PCP was shown for the first time here to be degraded under anaerobic condition created in a mediator – less single – chamber air cathode MFC. The response surface methodology (RSM) based on central composite rotatable design (CCRD) was employed for the optimization of PCP degradation and electricity generation in microbial fuel cell simultaneously. The predicted optimum conditions of PCP – glucose concentration (A), temperature (B) and pH (C) were at 70-2500 mg/L , 20^{o}C and 7.5 , respectively , resulting in PCP degradation , power density and coulombic efficiency of 73 % , 23.08 W/m^2 and 49 % , respectively. The correlation coefficients obtained for all of the responses justify an excellent correlation between the independent variables. Dominant bacteria similar to the anaerobic *Desulfobacterium aniline*, *Actinomycetes*, *Streptacidiphilus*, *Rhodococcus erythropolis*, *Amycolatopsis* and *Gordonia*, were found on the anodic biofilm. These results demonstrate efficient degradation of PCP in MFC and generation of electricity simultaneously.

References

Adour L.; Arbia W.; Amrane A.; Mameri N. (2008) Combined use of waste materials-recovery of chitin from shrimp shells by lactic acid fermentation supplemented with date juice waste or glucose. J. Chem. Techno. Biotechn.83: 1664-1669.

Auta M.; Hameed B.H. (2011) Optimized waste tea activated carbon for adsorption of Methylene Blue and Acid Blue 29 dyes using response surface methodology. Chem. Engin. J. 175: 233–243.

Bezerra M.A.; Santelli R.E.; Oliveira E.P.; Villar L.S.; Escaleira L.A. (2008) Response surface methodology (RSM) as a tool for optimization in analytical. Talanta, 76: 965–977.

Box G.E.P.; Wilson K.B. (1951) On the experimental attainment of optimum conditions. J. Royal Stat. Soc. Ser. 13(1): 1 – 38

Bryant F.O.; Hale D.D.; Rogers J.E. (1991) Regiospecific dechlorination of pentachlorophenol by dichlorophenol-adpated microorganisms in freshwater, anaerobic sediment slurries. Appl. Environ. Microb. 57: 2293-2303.

Butler C.S.; Clauwaert P.; Green S.J.; Verstraete W.; Nerenberg R. (2010) Bioelectrochemical perchlorate reduction in a microbial fuel cell. Environ. Sci. Technol. 44: 4685–4691.

Chang B.V.; Liou M.B.; Yuan S.Y.; Pan T.M. (1995) Anaerobic degradation of chlorophenols by 2,4-dichlorophenol-adapted microbial communities at different concentrations. Oceanogra. Liter. Revi. 42: 1022 – 1030.

Chang B.V.; Zheng J.X.; Yuan S.Y. (1996) Effect of alternative electron donors, acceptors and inhibitors on pentachlorophenol dechlorination in soil. Chemosph. 33: 313–320.

DC M. (2001)Design and analysis of experiment. 5^{ed}. New York: John Wiley and Sons.

Dougherty R.C. (1997) Human exposure to pentachlorophenol. Pentachlorophenol: Chemistry, Pharmac. Environ. Toxicol. 53–65.

Drzyzga O.; Navarro-Llorens J.M.; de las Heras L.F.; Fern-dez E.G.; Perera, J. (2009) Gordonia cholesterolivorans sp. nov., a cholesteroldegrading actinomycete isolated from sewage sludge. Int. J. Syst. Evol. Microbiol. 59: 1011–1015.

Duff S.J.B.; Kennedy K.J.; Brady A.J. (1995) Treatment of dilute phenol/PCP wastewaters using the upflow anaerobic sludge blanket (UASB) reactor. Wat. Rese. 29: 45–651.

Escher B.I.; Snozzi M.; Schwarzenbach R.P. (1996) Uptake, speciation and uncoupling activity of substituted phenols in energy transducing membranes. Environ. Sci. Techno. 30: 3071–3079.

Farrell A.; Quilty B. (1999) Degradation of mono-chlorophenols by a mixed microbial community via a meta-cleavage pathway. Biodegradation 10: 353–362.

Field J.A.; Sierra-Alvarez R. (2008) Microbial degradation of chlorinated phenols. Rev. Environ. Sci. Biotechnol. 7: 211–241.

Gan C.Y.; Latiff A.A. (2011) Optimization of the solvent extraction of bioactive compounds from Parkia speciosa pod using response surface methodology. Food Chem. 124: 1277–1283.

Ghani Z.A.; Ishak M.A.M.; Ismail K. (2010) Direct liquefaction of Mukah Balingian lowrank Malaysian coal: optimization using response surface methodology. Asia Paci. J. Chem. Engine. 6: 581 - 588.

Ghani Z.A; Khamil I.N.M.; Isa M.R.M; Jamaluddin M.A.; Ishak M.A.M.; Ismail K. (2011) Pyrolysis of Jatropha curcas l. Husk: optimization solid, liquid and gas yield by using response surface methodology (RSM). In: Proceedings of 3rd international symposium & exhibition in sustainable energy & environment (ISESEE). The Research and Innovations in Sustainable Energy (RISE) Center of Institute of Science (IOS) Universiti Teknologi MARA (UiTM).

Goswami M.; Shivaraman N.; Singh R.P. (2002) Kinetics of chlorophenol degradation by benzoate-induced culture of Rhodococcus erythropolis M1. World J. Microbiol. Biotechnol. 18: 779–783.

Halim S.F.A.; Kamaruddin A.H.; Fernando W.J.N. (2009) Continuous biosynthesis of biodiesel from waste cooking palm oil in a packed bed reactor: optimization using response surface methodology (RSM) and mass transfer studies. Bioresou. Techno. 100: 710 -716.

Hiroshi T.; Masasumi K.; Isao S. (1996) Anaerobic degradation of pentachlorophenol (PCP) in biological expanded-bed reactor. Wat. Sci. Techno. 34: 335–344.

Huang L.P.; Chen J.W.; Quan X.; Yang F.L. (2010) Enhancement of hexavalent chromium reduction and electricity production from a biocathode microbial fuel cell. Bioproc. Biosyst. Eng. 33: 937–945.

Huang L.P.; Gan L.L.; Zhao Q.L.; Loagn B.E.; Lu H.; Chen G.H. (2011b) Degradation of pentachlorophenol with the presence of fermentable and non-fermentable cosubstrates in a microbial fuel cell. Bioresour. Technol. 102: 8762–8768.

Huang L.P.; Regan J.M.; Quan X. (2011a) Electron transfer mechanisms, new applications, and performance of biocathode microbial fuel cells. Bioresour. Technol. 102: 316–323.

Karn S.K.; Chakrabarti S.K.; Reddy M.S. (2011) Degradation of pentachlorophenol by Kocuria sp. CL2 isolated from secondary sludge of pulp and paper mill. Biodegradation 22: 63–69.

Khayet M.; Zahrim A.Y.; Hilal N. (2011) Modelling and optimization of coagulation of highly concentrated industrial grade leather dye by response surface methodology. Chem.l Engine. J. 167: 77-83.

Kraigher B.; Mandic-Mulec I. (2011) Nitrification activity and community structure of nitrite-oxidizing bacteria in the bioreactors operated with addition of pharmaceuticals. J. Hazard. Mater. 188: 78–84.

Lee J.; Lee T.K.; Loffler F.E.; Park J. (2011) Characterization of microbial community structure and population dynamics of tetrachloroethene-dechlorinating tidal mudflat communities. Biodegradation 22: 687–698.

Li Z.; Yang S.; Inoue Y.; Yoshida N.; Katayama A. (2010) Complete anaerobic mineralization of pentachlorophenol (PCP) under continuous flow conditions by sequential combination of PCP-dechlorinating and phenol-degrading consortia. Biotechnol. Bioeng. 107: 775–785.

Liu H.; Logan B.E. (2004) Electricity Generation Using an Air-Cathode Single Chamber Microbial Fuel Cell in the Presence and Absence of a Proton Exchange Membrane. Environ. Sci. Technol. 38:4040 – 4046.

Liu Y.; Climent V.; Bern A.; Feliu J.M. (2011) Effect of temperature on the catalytic ability of electrochemically active biofilm as anode catalyst in microbial fuel cells. Electroanalysis 23: 387–394.

Lovley D.R.; Nevin K.P. (2011) A shift in the current: new applications and concepts for microbe-electrode electron exchange. Curr. Opin. Biotechnol. 22: 1 – 8.

Lovley D.R.; Phillips E.J.P. (1988) Novel mode of microbial energy metabolism: organic carbon oxidation coupled to dissimilatory reduction of iron or manganese. Appl. Environ. Microbiol. 54: 1472–1480.

Lu P.; Metcalf R.L.; Cole L.K. (1997) The environmental fate of 14Cpentachlorophenol in laboratory model ecosystems. Pentachlorophenol: Chemistry. Pharmaco. Environ. Toxico. 53–65.

Mikesell M.D.; Boyd S.A. (1985) Reductive dechlorination of the pesticides 2,4-D, 2,4,5-T, and pentachlorophenol in anaerobic sludges. J. Environ. Qual. 14: 337 – 345.

Mohn W.W. (1992) Limited degradation of chlorophenols by anaerobic sludge granule. Appl. Environ. Microb. 58: 365–367.

Myers R.H.; Montgomery D.C. (2002) Response surface methodology: Process end product optimization using designed experiments. 2nd ed. John Wiley and Sons, New York.

Pham H.T.; Boon N.; Marzorati M.; Verstraete W. (2009) Enhanced removal of 1,2-dichloroethane by anodophilic microbial consortia. Water Res. 43: 2936–2946.

Pieper D.H.; Martins dos Santos V.A.P.; Golyshin P.N. (2004) Genomic and mechanistic insights into the biodegradation of organic pollutants. Curr. Opin. Biotech. 15: 215–224.

Piringer G.; Bhattacharya S.K. (1999) Toxicity and fate of pentachlorophenol in anaerobic acidogenic system. Wat. Rese. 33: 2674–2682.

Prasad K.N.; Hassan F.A.; Yang B.; Kong K.W.; Ramanan R.N.; Azlan A. (2011) Response surface optimization for the extraction of phenolic compounds and antioxidant capacities of underutilized Mangifera pajang Kosterm. Peels. Food Chem. 128: 1121–1127.

Rabaey K.; Read S.T.; Clauwaert P.; Freguia S.; Bond P.L.; Blackall L.L.; Keller J. (2008) Cathodic oxygen reduction catalyzed by bacteria in microbial fuel cells. ISME J. 2: 519–527.

Rao M.S.; Mu_noz J.; Stevens W.F. (2000) Critical factors in chitin production by fermentation of shrimp biowaste. Appl. Microbio. and Biotechn., 54: 808-813.

Santos S.C.C.; Alviano D.S.; Alviano C.S.; Padula M.; Leitao A.C.; Martins O.B.; Ribeiro C.M.S.; Sassaki M.Y.M.; Matta C.P.S.; Bevilaqua J.; Sebastian G.V.; Seldin L. (2006) Characterization of Gordonia sp. strain F.5.25.8 capable of dibenzothiophene desulfurization and carbazole utilization. Appl. Microbiol. Biotechnol. 71: 355–362.

Shen D.S.; Liu X.W.; Feng H.J. (2005) Effect of easily degradable substrate on anaerobic degradation of pentachlorophenol in an upflow anaerobic sludge blanket (UASB) reactor. J. Haz. Mat. B119: 239–243.

Tan I.A.W.; Ahmad A.L.; Hameed B.H. (2008) Optimization of preparation conditions for activated carbons from coconut husk using response surface methodology. Chem. Engine. J. 137:462 - 470.

Togna M.T.; Kafkewitz D.; Armenante P.M. (1995) Rapid dehalogenation of 2,4,6-trichlorophenol at alkaline pH by an anaerobic enrichment culture. Lett. Appl. Microb. 20: 113–116.

Vargas A.M.M.; Garcia C.A.; Reis E.M.; Lenzi E.; Costa W.F.; Almeida V.C. (2010) NaOH – activated carbon from flamboyant (Delonix regia) pods: Optimization of preparation conditions using central composite rotatable design. Chem. Engin. J. 162: 43–50.

Wei F.S. (2002)Water and wastewater monitoring analysis method, 4th ed. Publishing House of Environmental Science of China, Beijing.

Wilson G.J.; Khodadoust A.P.; Suidan M.T.; Brenner R.C. (1997) Anaerobic/aerobic biodegradation of pentachlorophenol using

GAC fluidized bed reactors: optimization of the empty bed contact time. Wat. Sci. Techno. 36: 107–115.

Wrighton K.C.; Virdis B.; Clauwaert P.; Read S.T.; Daly R.A.; Boon N.; Piceno Y.; Andersen G.L.; Coates J.D.; Rabaey K. (2010) Bacterial community structure corresponds to performance during cathodic nitrate reduction. ISME J. 4: 1443–1455.

Ye F.X.; Shen D.S.; Feng X.S. (2004) Anaerobic granule development for removal of pentachlorophenol in an upflow anaerobic sludge blanket (UASB) reactor. Proce. Biochem. 39: 1249–1256.