Journal of Environmental Management 181 (2016) 343–352

Contents lists available at ScienceDirect

Journal of Environmental Management

journal homepage: www.elsevier.com/locate/jenvman

Research article

Modeling BOD and COD removal from Palm Oil Mill Secondary Effluent in floating wetland by *Chrysopogon zizanioides* (L.) using response surface methodology

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A R T I C L E I N F O

Article history:
Received 10 December 2015
Received in revised form
22 June 2016
Accepted 27 June 2016

Keywords:
Biological oxygen demand (BOD)
Chemical oxygen demand (COD)
Vetiver
Palm Oil Mill Effluent (POME)
Wastewater
Response surface methodology (RSM)

A B S T R A C T

While the oil palm industry has been recognized for its contribution towards economic growth and rapid development, it has also contributed to environmental pollution due to the production of huge quantities of by-products from the oil extraction process. A phytoremediation technique (floating Vetiver system) was used to treat Palm Oil Mill Secondary Effluent (POMSE). A batch study using 40 L treatment tanks was carried out under different conditions and Response Surface Methodology (RSM) was applied to optimize the treatment process. A three factor central composite design (CCD) was used to predict the experimental variables (POMSE concentration, Vetiver plant density and time). An extraordinary decrease in organic matter as measured by BOD and COD (96% and 94% respectively) was recorded during the experimental duration of 4 weeks using a density of 30 Vetiver plants. The best and lowest final BOD of 2 mg/L was obtained when using 15 Vetiver plants after 13 days for low concentration POMSE (initial BOD = 50 mg/L). The next best result of BOD at 32 mg/L was obtained when using 30 Vetiver plants after 24 days for medium concentration POMSE (initial BOD = 175 mg/L). These results confirmed the validity of the model, and the experimental value was determined to be quite close to the predicted value, implying that the empirical model derived from RSM experimental design can be used to adequately describe the relationship between the independent variables and response. The study showed that the Vetiver system is an effective method of treating POMSE.

1. Introduction

Malaysia has the second largest number of palm oil mills in the world after Indonesia (Abdullah and Sulaiman, 2013; Rupani et al., 2010). Amongst all wastes produced, researchers have concluded that Palm Oil Mill Effluent (POME) is the most difficult waste to handle due to the high volume generated (Madaki and Seng, 2013) and difficulties in handling its treatment (Madaki and Seng, 2013; Rupani et al., 2010). During the processing of POME, more than 70% (by weight) of the processed fresh fruit bunches usually remains as oil palm wastes (Prasertsan and Prasertsan, 1996).

There are about 430 palm oil mills in Malaysia that produce about 18.9 million tonnes of crude palm oil obtained from 92.9 million tonnes of fresh fruit bunches, with the assumption that the ratio of fresh fruit bunches processed to POME generated is 1:1.5 the total POME generated was about 139.35 million tonnes (Noorshamsiana et al., 2013).

POME has been identified as one of the main sources of water pollution in Malaysia due to the resulting high biochemical oxygen demand (BOD) and chemical oxygen demand (COD). Palm Oil Mill Secondary Effluent (POMSE), the product of secondary treatment of POME, is treated in ponds, using initial steps such as cooling and acid ponding followed by subsequent steps using anaerobic-aerobic treatments. POMSE is characterized by its thick, brownish color, higher pH (7–9 pH), but has a lower BOD and COD effluent as

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http://dx.doi.org/10.1016/j.jenvman.2016.06.060
0301-4797/

compared to POME. Although the industry claims that POMSE is properly treated with the pond system, open tank digesters and extended aeration systems, this conventional system has often been found to exceed the standard discharge. The main problems related to anaerobic treatment are long retention time, slow start up (granulating reactors), the production of greenhouse gases and the large area required for conventional digesters (Borja et al., 1996; Chan et al., 2010; Metcalf, 2003). Based on the new environmental challenges facing palm oil mills, there is an urgent need for the palm oil mills to explore and take advantage of the current options and alternatives to improving their environmental performance.

Today, constructed wetlands (CWs) for wastewater treatment represent innovative and promising solutions for environmental protection, placing them in the overall context of the need for low-cost and sustainable wastewater treatment systems in developing countries (Babatunde, Zhao, O'neill, & O'Sullivan, 2008; Vymazal, 2010). CWs have been successfully used to reduce environmental pollution by removing a wide range of pollutants from wastewater such as organic compounds, suspended solids, pathogens, metals, and nutrients (Gikas et al., 2013; Haberl et al., 1995; Kadlec and Wallace, 2008; Ranieri et al., 2013). Phytoremediation is an emerging, cost effective, aesthetically pleasing, low cost and suitable solution for many environmental problems across the world (Macek et al., 2004; Paz-Alberto and Sigua, 2013; Truong et al., 2010).

One of the phytoremediation methods for wastewater treatment is Floating Treatment Wetlands (FTWs) which is a novel treatment concept that employs rooted, emergent macrophytes (such as: Vetiver, Water hyacinth, Typha, etc.) growing on floating platforms rather than rooted in the sediments (Fonder and Headley, 2011; C. C. Tanner and Headley, 2011). One of the main advantages of using floating wetlands as a phytoremediation method is the simplicity of its implementation. No highly delineated design is needed for these wetlands. The floating plants facilitate the uptake of nutrients and pollutants irrespective of the water depth or area shape, implying that existing ponds at palm oil mills can be used directly without the need to build costly new wetlands.

In FTWs, the plant roots are not in contact with the benthic sediments or soil and can access nutrients contained within the floating platforms and in the water column (Kadlec and Wallace, 2008). Beneath the floating platforms, a network of roots, rhizomes, and the hanging root biofilm provides a biologically active surface area for the biochemical transformation of contaminants and physical processes such as filtering and entrapment of particulates (Kyambadde et al., 2004; Li et al., 2009).

Few phytoremediation treatment trials of inorganic contaminants in constructed wetlands have been reported such as: The Lorong Halus Wetland in Singapore is probably the biggest subsurface flow constructed wetland in the world, a wetland was established to treat leachate from an old landfill before it was released to a water reservoir. Vetiver Grass is one of three species used and it was evaluated and found to be the most effective and suitable of the three species tested (Truong and Truong, 2013). A pilot scale study in a subsurface flow treatment wetland was done by Anning et al. (2013) and showed a great potential of the aquatic macrophyte southern cattail (Typha domingensis) for the phytoremediation of water contaminated with mercury.

Application of Vetiver grass (*Chrysopogon zizanioides* L.), for wastewater treatment is a new and innovative phytoremedial method. Vetiver can be used to treat industrial and domestic wastewater due to its exceptional absorption ability and its capacity to tolerate excessive levels of nutrients (Truong, 2008; Wagner et al., 2003).

Response surface methodology (RSM) is an analytical tool used to establish the optimum conditions for a multi-variable structure and has been useful for optimizing wastewater treatment protocols. Conventional optimization methods are "one-factor-at-a-time" techniques. This approach often fails to identify the variables that give rise to the optimum response because the effects of factor interactions are not taken into account in such procedures (Deepak et al., 2008). Modeling and displacing experimental conditions are conducted using linear or polynomial functions to describe the system under study. RSM has the ability to determine the relationship and the interaction between the independent variable and various response variables based on the desired criteria. Moreover, a fewer number of experimental trials will be needed to evaluate the interaction if RSM is applied. Thus, optimizing an experimental process becomes less time consuming (Fard Masoumi et al., 2013; Kalantari et al., 2014; Montgomery, 2008; Sohrabi et al., 2014).

To date, no attempt has been made to model POME and POMSE treatment by Vetiver grass on floating wetlands. The main objective of this study was to evaluate Vetiver grass with respect to its ability to reduce BOD and COD in aeration condition and to optimize the POMSE treatment with Vetiver grass by response surface methodology. The removal of COD and BOD were chosen as the dependent output variables, POMSE concentration and Vetiver density were chosen as the influence factors.

2. Methodology

2.1. Sample collection

POMSE samples were collected from a nearby palm oil mill in Labu, Negeri Sembilan, Malaysia (2°47.08′ N and 102°30.25′E). POMSE was collected from the final discharge pond. Collected and analyses were done on the same day. Table 1 shows the characteristics of POMSE generated from the Labu palm oil mill.

2.2. Classification of POMSE in experiment

The focus of this research is to treat POMSE, with BOD ranging from 50 to 350 mg/L. Therefore, fresh POMSE collected from the mill was diluted to suit the various stages of this experiment. The experiments were conducted with three POMSE concentrations (Table 2).

2.3. Preparation of vetiver seedlings

Vetiver (*Chrysopogon zizanioides* L.) planting stock was obtained from a commercial nursery, Humibox (M) Sdn. Bhd, Malaysia. To adapt to the new environment, the Vetiver slips were first transplanted to a temporary hydroponic nursery. They were kept in large containers (40 L) for 5 weeks until an adequate number of new roots and shoots were obtained (Fig. 1). After five weeks Vetiver slips with roughly the same size were selected from the hydroponic solution and shoots were cut back to approximately 20 cm height to

Table 1
Characteristics of POMSE discharged from last pond.

Parameter	POMSE	[a]DOE standard
pH	7.5	7.2
TSS (mg/L)	790	400
COD (mg/L)	750	–
BOD (mg/L)	350	100
BOD/COD	0.46	–
Color (ADMI)	3750	200
TN (mg/L)	450	200
Ammonia (mg/L)	300	100
Oil and Grease (mg/L)	15	50

[a] Department of Environment (DOE).

Table 2
POMSE concentration ratio for dilution.

Treatment	Mill POMSE %	Water %
Low	10	90
Medium	50	50
High	100	0

reduce transpiration and roots were cut back to a 10 cm length (Fig. 1). There was no report in the literature indicating the numbers of plants suitable for research similar to this study, so 5, 15 and 30 plants were used in this study. Five plants were chosen as the minimum and 30 plants were considered as the maximum. Due to rapid growth and increased Vetiver biomass production, using more than this density could have resulted in sinking the floating pontoon into the effluent.

2.4. Treatment tank and polystyrene cover configuration

In floating wetlands, plants grow on floating platforms rather than rooted in soil or sediment, so water depth of the ponds is not a concern, and the platforms are unlikely to be affected by fluctuations in water levels (Chang et al., 2014; Headley et al., 2008). In this study, the free floating system was selected for the treatments because floating plants have higher removal efficiencies as compared to rooted emergent plants. This may be attributed to the fact that they float freely in aquatic systems and cover more area for absorption as compared to the fixed emergent plants (Kumari and Tripathi, 2014). A rectangular shaped tank was used with the following dimensions: 30 cm × 30 cm × 50 cm (length × width × depth) with a nominal volume of 45 L, but 40 L was chosen as the experimental volume. The whole experiment was implemented under a clear horticultural plastic shelter that excluded rainfall (Sooknah and Wilkie, 2004). Polystyrene sheets, with a dimension of 30 cm × 30 cm × 5 cm (length × width × thickness), were used as the platform to support the Vetiver plants. They were placed on the container's surface. Vetiver slips were planted into holes in the polystyrene platform with their roots submerged in POMSE.

2.5. Aeration system

Two sets of experiments were conducted: one under aerobic and another under anaerobic conditions to determine the effects of aeration on Vetiver growth. Under natural wetland conditions, oxygen is supplied to the water body via atmospheric diffusion, or by direct transfer through the plant's aerenchyma tissues (Kumari and Tripathi, 2014; Moorhead and Reddy, 1988; Zhang et al., 2010). In the conventional aerobic wastewater treatment system, dissolved oxygen (DO) concentrations are maintained from 1 to 3 mg/L (Metcalf, 2003). In contrast, oxygen concentrations in floating wetlands with high organic loading matter such as in the presence of POME frequently are less than 0.5 mg/L. Due to the low oxygen concentration, the provision of 3 mg/L oxygen to the solution continuously in this experiment is essential to maintain aerobic bacterial activity. The aeration was carried out by using an aquarium pump with a porous stone diffuser placed at the bottom of tank.

2.6. pH adjustment test

In order to monitor the effect of the variation of pH on the performance of Vetiver, experiments were conducted in the laboratory at different pH levels (levels adjusted with dilute Sodium Hydroxide (NaOH) and Sulfuric acid (H_2SO_4)) at 25 °C. pH was measured using a HANNA™ (HI 8424) pH meter.

2.7. Experimental design

Two sets of experiments were conducted: one under aerobic and another under anaerobic conditions to determine the effects of aeration on Vetiver growth. For each set, three POMSE concentration levels were used: low concentration (LCP); medium concentration (MCP); and high concentration (HCP). These were combined with three Vetiver plant densities: 5 slips (V5); 15 slips (V15); and 30 slips (V30) per container. The control treatment did not have any Vetiver plants. Table 3 summarizes the experimental design. All experiments were carried out in triplicate.

2.8. Sampling and analysis

The treatments were evaluated by taking samples every week, in mid-morning, then analyzing them for BOD and COD. Samples were obtained by dipping a 100 ml graduated pipette at three locations inside the container then combining them into one sample. All POMSE sampling equipment was acid rinsed followed by distilled water flushing prior to sampling of each tank. POMSE in each container was sampled 5 times over the 4-week experimental period: on the first day; weeks 1; 2; 3; and 4.

BOD was determined using a HACH BOD Trak™ instrument. BOD nutrient pillows were added to each of the bottles along with 20 ml of POMSE. The bottles were then sealed and incubated on the BOD Trak™ instrument, which automatically monitored the BOD continually over 3 days. The samples were continually stirred at 30 °C using magnetic stir bars.

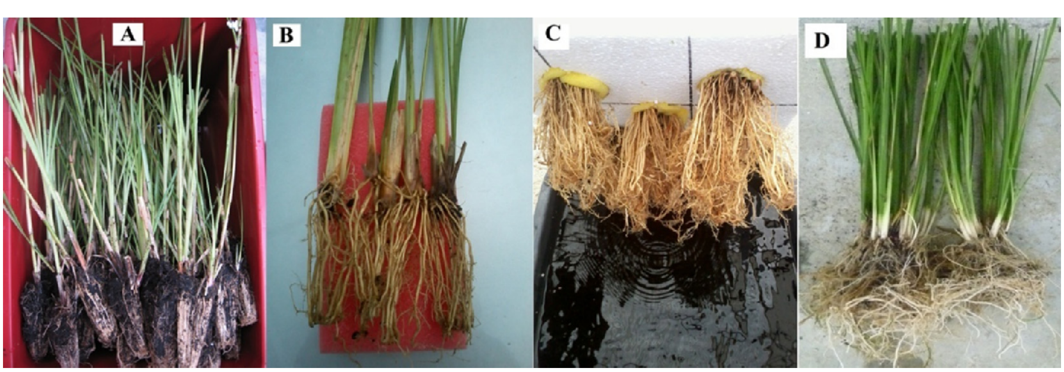

Fig. 1. (A): Vetiver slips initial day (B): Vetiver slips washed to remove debris (C): Vetiver slips in hydroponic solution and (D): Vetiver slips after five weeks growing in hydroponic solution ready for experimentation.

Table 3
Experimental design.

Vetiver slips density (number)				POMSE concentration
V 5	V 15	V 30	Without Vetiver (Control)	Low conc. POMSE (LCP) Medium conc. POMSE (MCP) High conc. POMSE (HCP)

To measure COD concentration, samples were digested in a preheated HACH COD reactor at 150 C for 2 h before the absorbance measurement was carried out with a UV spectrophotometer (DR/6000, HACH Company, USA) at a wavelength of 600 nm. All samples were measured in triplicates.

2.9. RSM experimental design

RSM includes a group of empirical techniques devoted to the estimate of relations existing between a group of experimental factors and the measured responses. Understanding of the process variables under investigation is necessary to achieve a more practical model. Central Composite Face Centered (CCF) which is a type of central composite design (CCD) for three independent variables was employed to determine the effect value of POMSE concentration (10, 50 and 100%, X_1), Vetiver slip density (0, 5, 15 and 30 slips, X_2) and time (1, 2, 3 and 4 weeks, X_3), on two response variables: COD (Y_1) and BOD (Y_2). The experiment had 20 runs, with 6 center points and 4 axial points, and $\alpha = 1$. All experiments were carried out in triplicate and the mean values are reported. One of the data have been deleted from the model due to it was detected as outlier by statistical evaluation. The maximum deviation was found to be ±2%. Removal efficiencies of the treatment system were calculated based on fallowing formula:

$$\%\text{Removal Efficiency} = \left(\frac{C_{inf} - C_{eff}}{C_{inf}}\right) \times 100 \quad (1)$$

where C_{inf} is initial parameter concentration and C_{eff} is final parameter concentration.

3. Results and discussion

3.1. Vetiver growth in POMSE with and without aeration

Aeration facilitates aerobic degradation of organic materials by maintaining adequate oxygen concentration in the wastewater. Aeration is also an important factor that influences root and plant growth as oxygen is essential for cell growth and function. If not available or limited in the rooting medium, severe plant injury or death will occur. The energy required for root growth and ion absorption is derived from the “respiration” process that requires oxygen. Without adequate oxygen to support respiration, water and ion absorption cease and roots die (Jones, 2014).

Preliminary results demonstrate that anaerobic conditions negatively affect Vetiver growth (Fig. 2) as the Vetiver leaves in the anaerobic treatments started to brown off by the third day of the experiment and dried up by the end of the fifth day. Since Vetiver did not survive in the anaerobic experiment, this system was not monitored during the rest of the study. However, several earlier studies have been conducted to improve the quality of industrial effluent using aerated systems (Dong et al., 2012; Kumari and Tripathi, 2014; Zhang et al., 2010). In this study 3 mg/L of oxygen was added to the system to promote bacterial activity. Fig. 2 compares Vetiver growth in anaerobic and aerobic conditions, and Fig. 3 shows healthy root growth under aerobic conditions and dead roots under anaerobic conditions.

Although, Vetiver plants were affected by aeration, they were not affected by high COD and BOD in the high concentration treatment. Other studies have reported the death of macrophytes due to parameters such as: high ammonia concentration and high COD and BOD. C. C. Tanner (1995) reported root death in plants growing in piggery wastewater that had 222 mg/L ammonia concentrations. Paredes et al. (2007) reported the death of *Juncus effusus* when NO_3 and NH_4 concentrations were 91 mg/L and 156 mg/L respectively in laboratory-scale models with anaerobic ammonium oxidizing bacteria. In another study Roongtanakiat et al. (2003) reported death of Vetiver grass in the treatment of leachate due to the very elevated COD of 13,160 mg/L and BOD of 6607 mg/L Sooknah and Wilkie (2004), reported water lettuce and pennywort did not survive in undiluted dairy wastewater treatments.

3.2. Effect of pH

Fig. 4 shows that at a pH below 5, Vetiver performance (BOD removal) was less than 40%, 20% and 10% for low, medium and high concentration respectively. On the other hand when pH gradually increased, performance improved up to pH 7, but Vetiver performance was retarded by further increase in pH and at pH 12 (extreme alkalinity), the Vetiver treatment efficiency was reduced to zero. Therefore, it appears that a pH range from 5 to 9 is most suitable for treatment. As the pH of POMSE used in the current research was between 7 and 8, results obtained should be at the optimal efficiency.

3.3. Analysis of experimental data and prediction of performance of BOD and COD removal

Experimental data and the predicted values for BOD and COD removal percentage are presented in Table 4. The actual values are obtained from experiments and the predicted values are obtained from model fitting method. As shown in Fig. 5 the predicted values fit well to the actual values. The predicted responses obtained from RSM were compared to the actual responses, for verification of the predicted data.

3.3.1. Regression analysis for COD and BOD removal

The removal of COD depends on the combination of physical and microbial mechanisms. Because of the physical root filtration mechanism, solids could be filtered and trapped in the roots, thereby allowing for better biodegradation of organic solids. The high percentages removal for COD is caused by sedimentation of suspended solids and by decomposition processes in the POMSE.

A regression method was used to fit the quadratic model to the experimental data and to identify the relevant model terms. Regression data analysis then generated corresponding sets of coefficients for developing a model equation (Table 5). The regression model ANOVA was conducted to find the significance of the main and interacting effects of the removal process parameters. Our model *F* value of 76.72 and 41.06 for COD and BOD respectively implies the model is significant. There was only a 0.01% chance that

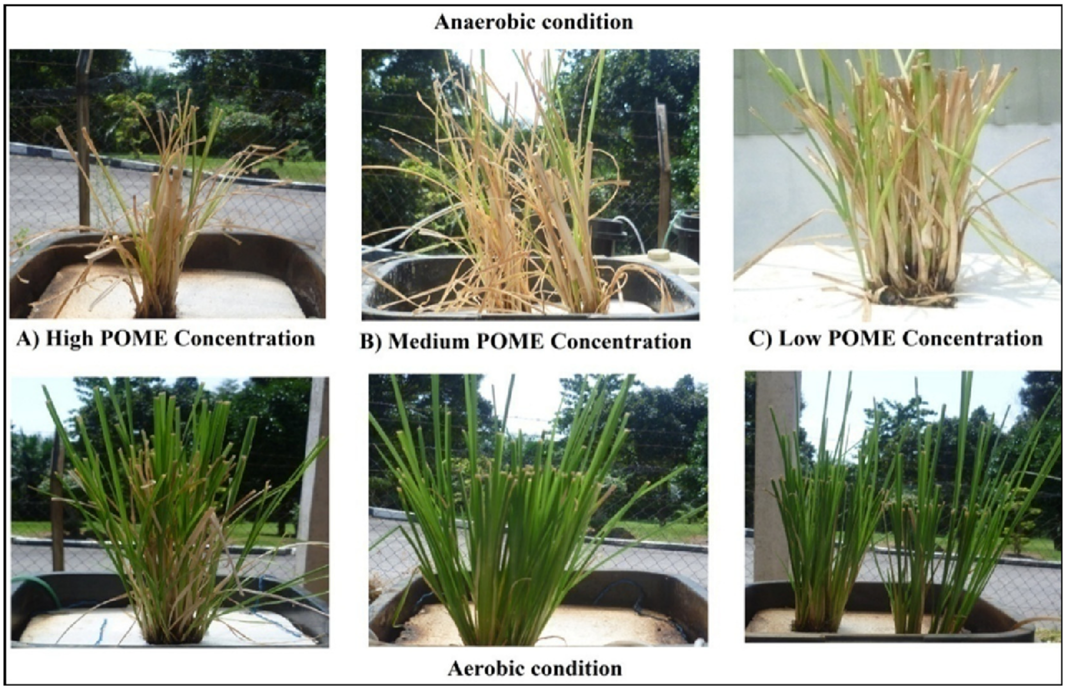

Fig. 2. (A): Vetiver growth in high POMSE concentration; (B) Vetiver growth in medium POMSE concentration and (C) Vetiver growth in low POMSE concentration (top photos are of an anaerobic condition and the lower photos are of an aerobic condition).

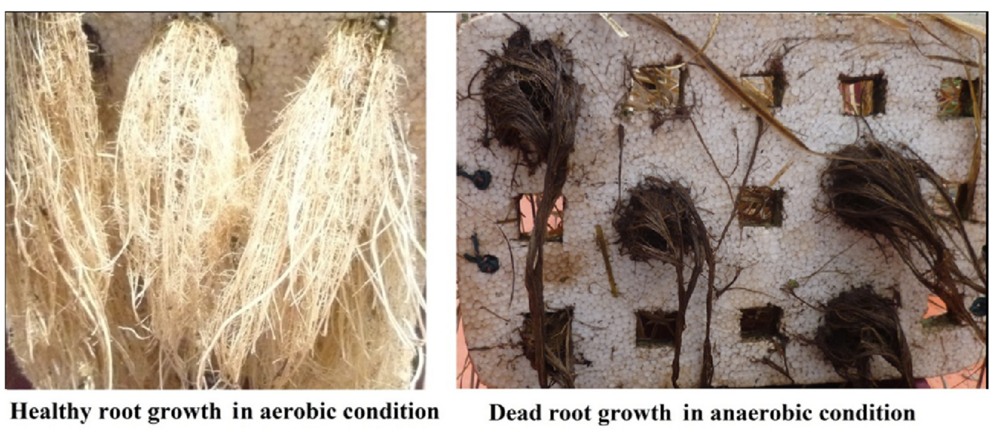

Fig. 3. Study of healthy and dead Vetiver roots in aerobic and anaerobic condition.

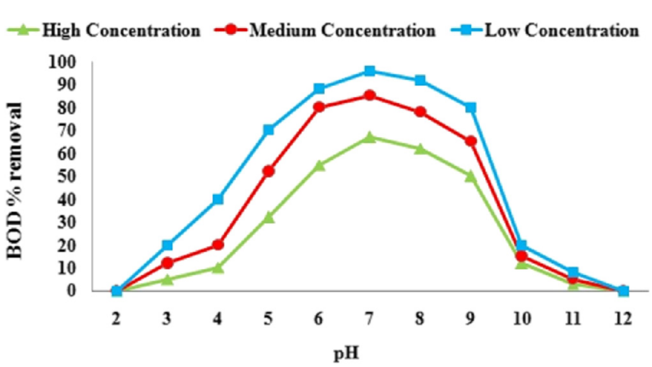

Fig. 4. Variation of pH on performance of Vetiver for BOD removal.

the "Model *F* value" occurred due to noise and most of variation in the response can be described by the regression equation for the model that is significant. The lack-of-fit *F* value of 4.13 and 2.16 for COD and BOD respectively implies there is a 7.06% and 21.14% chance that a lack-of-fit *F value* occurs due to noise.

The coefficient of determination (R^2) of the model is 0.987 for COD and 0.988 for BOD indicating that the model explains 98.7% and 98.8% of the response variability. The present R^2-value reflected a very good fit between experimental and predicted values. The adjusted determination coefficient (Adjusted R^2 COD = 0.974 and Adjusted R^2 BOD = 0.957) is also sufficiently high to confirm the model significance.

The final reduced model to predict the percentage of COD and BOD removal based on Vetiver system to treat POMSE is shown in Equation (2) and Equation (3). Negative values of coefficient estimates indicate negative influence of parameters on the reaction. It was observed that Vetiver density and time have significant effects to the reaction, and vetiver density has one of the biggest effects to response.

Table 4
Central composite design matrix, actual and predicted values of the BOD and COD removal.

Run	X_1	X_2	X_3	BOD % removal		COD % removal	
	Concentration (mg/L)	Vetiver slips density	Time (Week)	Actual	Predict	Actual	Predict
1	0[a]	0	0	64.45	61.01	65.00	58.62
2	−1[b]	1	1	96.00	96.48	94.40	93.71
3	1[c]	−1	−1	9.15	7.80	8.10	7.08
4	0	0	0	62.00	61.01	59.40	58.62
5	1	0	0	55.65	56.18	54.00	59.06
6	−1	−1	1	22.00	21.52	16.60	18.44
7	0	0	0	66.00	61.01	58.00	58.62
8	−1	1	−1	56.00	59.38	50.00	52.96
9	0	0	0	65.15	61.01	59.00	58.62
10	0	1	0	81.50	74.87	68.00	67.01
11	1	1	1	61.45	63.39	62.30	63.92
12	−1	0	0	92.80	93.55	71.00	69.52
13	0	0	0	56.00	61.01	59.00	58.62
14	1	−1	1	19.15	17.21	19.10	16.33
15	0	0	0	55.00	61.01	58.50	58.62
16	−1	−1	−1	4.00	0.13	3.60	0.96
17	0	0	−1	21.15	22.43	29.00	32.58
18	0	−1	0	14.30	22.21	15.10	19.67
19	1	1	−1	37.70	38.52	34.30	31.40

[a] Low Concentration.
[b] Medium Concentration.
[c] High Concentration.

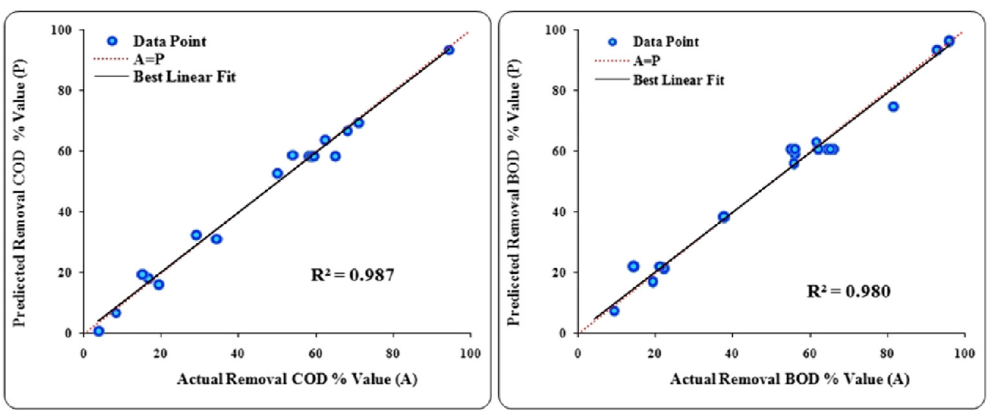

Fig. 5. Scatter plot of predicted removal % value versus actual removal % value from RSM experimental design.

$$Y_{COD} = 64.38 - 5.29X_1 + 24.90X_2 + 12.50X_3 - 6.29X_1X_2 - 2.6X_1X_3 + 5.82X_2X_3 + 6.27X_1^2 - 15.28X_2^2 - 19.77X_3^2 \quad (2)$$

$$Y_{BOD} = 65.10 - 19.70X_1 + 26.42X_2 + 11.63X_3 - 7.20X_1X_2 - 3.06X_1X_3 + 3.86X_2X_3 + 17.46X_1^2 - 10.23X_2^2 - 34.30X_3^2 + 13.41X_1X_2^2 \quad (3)$$

where Y = removal Percentage, X_1 = POMSE concentration, X_2 = Vetiver slips and X_3 = Time.

3.3.1.1. Response surface analysis for COD and BOD removal. The final equation derived from the regression analysis (Equations (2) and (3)) was then used to facilitate plotting of response surfaces. It is plotted to understand the interaction of the variables and locate the optimal level of each variable for maximal response. Each response surface plotted for COD and BOD removal represented the different combinations of two test variables at one time while maintaining the other variable at the center point values (coded level: 0). This graphic representation helps to visualize the effects of the combination of factors.

3.3.1.2. Interactive effect of COD and BOD concentration and vetiver density (X_1X_2). Microbial degradation has been identified as the major contributing factor for the removal of biodegradable organic matter (BOD) in wastewater. It has been demonstrated that biodegradation takes place when dissolved organic matter contacts the biofilm that is found on submerged plant stems, roots, surrounding soil or media via diffusion processes. Plants provide the medium for microbial degradation and convey oxygen to their rhizosphere for aerobic degradation (Idris et al., 2014).

The physical characteristics of the root itself play a major role in elemental ion uptake. The rooting medium and the elements in the medium determine to a considerable degree root appearance (Jones, 2014). Fig. 6 (A and D) shows the effect of COD and BOD concentration and Vetiver slips density on percentage removal of POMSE. The result showed that a decrease in POMSE concentration and the increase in Vetiver slips density improved percentage removal. Similar findings were also reported by Klomjek and Nitisoravut (2005), who noted a higher reduction in BOD

Table 5
Analysis of variance (ANOVA) and regression coefficients of COD and BOD percentage removal (Quadratic model).

Source	COD				BOD		
	Mean square	*F* value	*p* value		Mean square	*F* Value	*p* Value
Intercept	–	–	–	Intercept	–	–	–
X_1	348.24	21.8	0.0012	X_1	759.37	23.02	0.0014
X_2	6161.62	385.75	<0.0001	X_2	6934.72	210.18	<0.0001
X_3	1305.45	81.73	<0.0001	X_3	1129.87	34.24	0.0004
X_1X_2	384.02	24.04	0.0008	X_1X_2	416.57	12.63	0.0075
X_1X_3	34.65	2.17	0.1749	X_1X_3	74.89	2.27	0.1703
X_2X_3	276.79	17.33	0.0024	X_2X_3	122.04	3.7	0.0907
X_1^2	89.49	5.6	0.0421	X_1^2	660.27	20.01	0.0021
X_2^2	544.94	34.12	0.0002	X_2^2	239.41	7.26	0.0273
X_3^2	577.84	36.18	0.0002	X_3^2	1726.51	52.33	<0.0001
–	–	–	–	$X_1X_2^2$	283.28	8.59	0.0190
Model	1225.49	76.72	<0.0001[a]	Model	1354.72	41.06	<0.0001
Residual	15.97	–	–	Residual	32.99	–	–
Lack of fit	27.59	4.13	0.076[b]	Lack of fit	49.65	2.16	0.2114
Pure error	6.68	–	–	Pure error	23	–	–
R-squared	0.987	Standard deviation	4	R-squared	0.988	Standard deviation	5.74
Adjusted R^2	0.974	Coefficient of variation %	8.59	Adjusted R^2	0.957	Coefficient of variation %	11.62
Adequate precision	31.987	PRESS[c]	1506.2	Adequate precision	22.105	PRESS[c]	1961.89

Note:
X_1 = POMSE concentration (mg/L), X_2 = Vetiver density (Slips), X_3 = Time (Week).
[a] Model F-value is significant at "Prob > F" less than 0.05.
[b] Lack of Fit value is not significant relative to pure error.
[c] PRESS is Predicted Residual Error of Sum of Squares.

(72.4–78.9%) in units with plants as compared to the unplanted one. Abou-Elela and Hellal (2012) reported that high BOD removal values may be explained in wetland systems as settleable organic compounds were rapidly removed by deposition and filtration, while the other organic compounds were degraded both aerobically and anaerobically by the heterotrophic microorganisms depending on the oxygen concentration in the bed.

3.3.1.3. Interactive effect of COD and BOD concentration and time (X_1X_3). Response surface plot for the interaction between COD and BOD concentration (mg/L) and time was generated with a fixed Vetiver density of 15 slips. As shown in Fig. 6 (B and E), it was found that the removal percentage increased with increasing time up to 3 weeks. However, increasing the time beyond 3 weeks did not show any significant effect on the percentage removal. It is generally assumed that planted treatments outperform controls (without Vetiver) mainly because the Vetiver rhizosphere stimulates the microbial community density and activity by providing root surfaces for microbial growth, and a source of carbon compounds through root exudates (C. Tanner, 2001; Vymazal and Kröpfelová, 2009). Similar findings were reported by Liao et al. (2003); Njau and Mlay (2003) and Mishra and Tripathi (2008) which support the present observation which support the present observation. These findings are consistent with reports from Akratos and Tsihrintzis (2007), and Shah et al. (2014) Sehar et al. (2015) who found maximum BOD removal rates up to 90.0% at 10–14 days retention time.

3.3.1.4. Interactive effect of vetiver density and time for COD and BOD removal (X_2X_3). Fig. 6 (C and F) shows that an increase in both Vetiver density and time increased the percentage removal to a maximum value. A treatment with more Vetiver slips and longer retention time could result in increasing the removal up to higher levels. This indicated that the removal was greatly affected by retention time and Vetiver slips density.

3.4. Optimization by response surface methodology and validation for COD and BOD removal

Optimum value determination for control variables (factors) is one of the main objectives of RSM that can maximize or minimize a response over a certain region of interest. Having a 'good' fitting model is necessary to provide an adequate representation of the mean response because such a model is utilized to determine the value of the optimum (Khuri and Mukhopadhyay, 2010).

In order to determine the effects of three independent variables (POMSE concentration, Vetiver slip density and time) along with the predicted values for COD and BOD, a RSM was adopted using a central composite design for finding optimal conditions (Table 6). The optimum reaction parameters were different. Due to limitation of supply POMSE for validation set, a new set of experiments was carried out under the optimized recommended conditions and resulting responses was compared to the predicted values.

As shown in Table 6, different POMSE concentrations (maximum, medium and minimum) are compared at different Vetiver densities (maximum number and in ranges) and for different retention times (maximum and minimum). A new set of experiments was then carried out under the recommended conditions and resulting responses was compared to the predicted values.

The comparison was done based on Relative Standard Error (RSE). The RSE is the standard error expressed as a fraction of the estimate and is usually displayed as a percentage. Estimates with a RSE of 25% or greater are subject to high sampling error and should be used with caution.

Results show that minimum and maximum retention times were 11 and 28 days respectively. The results confirmed the validity of the model, and the experimental value were determined to be quite close to the predicted values implying that the empirical model derived from RSM experimental design can be used to adequately describe the relationship between the independent variables and response with RSE of less than 1.33%.

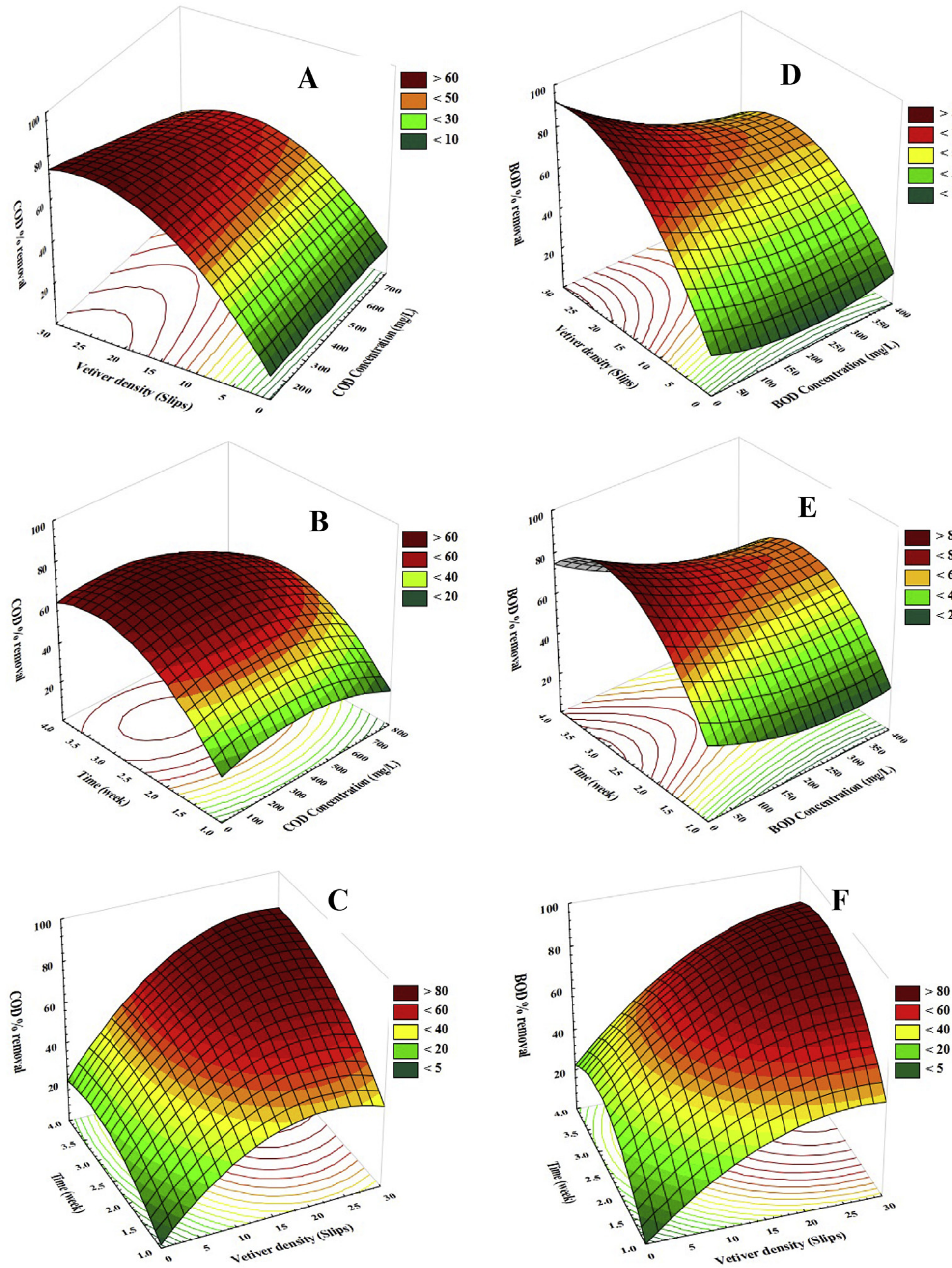

Fig. 6. (A) Response surface plot of COD (mg/L) versus Vetiver density (slips) (X_1X_2) on COD percentage removal as response; (B) COD (mg/L) versus Time (X_1X_3); (C) Vetiver slips density versus Time (X_2X_3); (D) Response surface plot of BOD (mg/L) versus Vetiver density (slips) (X_1X_2) on BOD percentage removal as response; (E) BOD (mg/L) versus Time (X_1X_3); (F) Vetiver slips density versus Time (X_2X_3).

Table 6
Optimum conditions derived by RSM for COD and BOD removal Percentage.

Optimum criteria				Independent variables				Removal (%)		RSE%
POME conc. (mg/L)	Vetiver slips (no.)	Time (day)	Removal %	Parameter	POME conc. (mg/L)	Vetiver slips (no.)	Time (day)	Removal % pre.	Removal % exp.	
Max	Max	Max	Max	BOD	350	30	27	68.01	67.45	0.82
				COD	750	30	27	65.91	66.50	0.90
Min	In range	Min	Max	BOD	50	15	13	95.99	95.62	0.39
				COD	115	15	11	64.71	64.30	0.63
Medium	Max	Max	Max	BOD	175	30	24	80.23	81.20	1.21
				COD	400	30	28	74.13	74.00	0.18
Medium	In range	Min	Max	BOD	175	15	13	62.40	62.20	0.32
				COD	400	15	12	55.75	55.01	1.33

3.5. A techno-economic assessment of the Vetiver system

Palm oil processing is a low cost activity and the applicability of a higher cost waste processing system will be unconvincing to palm oil millers. In comparing the Vetiver System method used in this study with other physical and chemical methods (Tank digestion and mechanical aeration, Physico-chemical and biological treatment, Coagulation-flocculation and Membrane technology) that have been used for POMSE treatment, it is obvious that the complexity of the latter methods and their high implementation costs are their main disadvantages. In contrast a phytoremediation system is a simple, low cost environmentally friendly method for use on existing ponds in palm oil mills. In terms of efficiency, phytoremediation using the Vetiver System is at least equal and often superior to existing methods that have been used for treatment of secondary POME.

4. Conclusion

Palm Oil Mill Secondary Effluent (POMSE), the product of secondary treatment of POME, is facing great difficulty in achieving effluent regulation of BOD 20 mg/L, as conventional treatment systems cannot attain satisfactory compliance set by the Department of Environment (DOE) Malaysia. This research aims at finding a sustainable low-cost polishing treatment that will benefit more than 400 palm oil mills in Malaysia. The phytoremediation method using Vetiver grass (*Chrysopogon zizanioides*) is a new and innovative polishing treatment used for POMSE. This study investigated the potential of Vetiver grass to treat POMSE in terms of BOD and COD reduction. The result showed that a significant decrease in organic matter such as BOD and COD was 96% and 94% respectively, for with an experimental duration of 4 weeks and using 30 Vetiver plants. The best and lowest final BOD of 2 mg/L was obtained for low concentration POMSE (initial BOD = 50 mg/L) using 15 Vetiver slips in 13 days. The second best outcome occurred when POMSE with an initial BOD of 175 mg/L (medium concentration) was reduced to a BOD of 32 mg/L after 24 days using 30 Vetiver slips. This significant achievement of obtaining acceptable results in less than 1 month would indicate an important breakthrough, as normal biological treatment using ponds need at least 2–3 months detention time. Using an increase in Vetiver density to 30 plants, and at 4 weeks operating time had indeed increased the percentage removal to a maximum value.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgement

The authors acknowledge the Malaysian Palm Oil Board (MPOB) for providing research fund and facilities.

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