

Investigation and optimization of a novel MAHB reactor for COD and lignin removal and methane production using response surface methodology (RSM) and artificial neural network (ANN)

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In this study, response surface methodology (RSM) and artificial neural network (ANN) were used to investigate and optimize COD and lignin removal and methane production rate in a novel modified anaerobic hybrid baffled (MAHB) reactor treating recycled paper mill effluent (RPME). Both feeding COD concentration and hydraulic residence time (HRT) are recognized as the two most important factors that affect COD and lignin removal and methane production rate. RSM analysis gives an optimum condition with HRT of 3.93 days and feeding COD concentration of 3020.88 mg L⁻¹ that yield COD removal efficiency of 97.42 %, lignin removal efficiency of 59.59 % and methane production rate of 8.07 L CH₄ day⁻¹ with desirability value of 0.897. From the analysis using ANN, results show a good agreement between experimental and ANN outputs for COD removal, lignin removal and methane production rate with R² values of 0.970, 0.9906 and 0.9545, respectively. These demonstrated that RSM and ANN were effective to assess and optimize the MAHB reactor system for COD and lignin removal and methane production, which provides a promising guide to further improvement of the system for potential applications.

Keywords: Modified anaerobic baffled reactor, Recycled paper mill effluent, RSM, ANN, Anaerobic digestion.

INTRODUCTION

The rise in environmental concerns related with the production of energy with CO₂ mitigation policies has renewed the interest in anaerobic digestion technologies. Anaerobic digestion (AD) is a series of bacteria events that convert organic matter in wastewater to methane (CH₄) and carbon dioxide (CO₂) that occurs in the absence of oxygen (O₂). This process has advantages in biogas recovery and waste stabilization. It has also been proven as a competent process in green technology for disposing crop residue, food waste, sewage sludge and animal manure [1,2]. Methane is a rich source of renewable energy which contributes to environmental conservation and sustainability by replacing the fossil fuel. This in turn govern the wide use of AD as an attractive means for paper mill wastewater treatment all around the globe while more and more new process configurations were developed [3].

The production of methane will be higher when several responses are combined in an optimized condition that will enhance the conversion process of organic matters. Among the factors that affect the methane fermentation are hydraulic retention time (HRT) and feeding COD concentration. If the process does not fall into the suitable range of optimized parameters, there is a tendency for

potential toxicity and digester failure. Mostly, the previous studies optimized the optimum conditions for COD and lignin removal and biogas production by the conventional method by changing one factor at a time. This is a method in which a single factor is varied while all the other factors are kept fixed at a specific condition. It is time consuming, laborious and difficult to reach the optimal conditions due to ignoring the interactions between variables. Hence, in this study D-optimal design of response surface methodology (RSM) and artificial neural network (ANN) are used as a beneficial technique for a statistical and mathematical process modeling and optimizing due to a more practical approach compared to the other as it arises from an experimental methodology. The results enable highlighting interactive effects among the variables and, eventually, it depicts the overall effects of the parameters on the process [4].

Several researchers described the application of RSM in wastewater treatment studies for multidisciplinary design of optimization study. Chou et al. [5] used CCD design of RSM to identify the optimum COD solubilization condition while treating palm oil mill effluent whereas Halim et al. [6] applied RSM based on central composite rotatable design to optimize the transesterification of waste cooking oil using continuous packed-bed reactor by manipulating two main variables which are substrate flow rate and packed-bed

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height. The results illustrated the effects of the operating variables along with their interactive effects on the responses. For better accuracy of the predicted model obtained by D-optimal design, artificial neural network (ANN) analysis was used. Previous study also showed that ANNs were successfully used to model the results of COD and lignin removal and biogas production with an expanded granular sludge-bed (EGSB) reactor [7] and an upflow anaerobic sludge blanket reactor (UASB) [8]. Holubar et al. [9] also used ANNs to control and model methane production from anaerobic continuously stirred tank reactors under different organic loading rates.

In the present study, a novel MAHB reactor was developed where each modified baffle has its own characteristics (form/shape) to facilitate a better contact and greater mixing of wastewater and anaerobic microorganisms [10]. This novel MAHB is a combination of regular suspended-growth and fixed biofilm systems. Furthermore, the treatment of this recycled paper mill effluent (RPME) is rarely studied using ABR. With RSM and ANN, the interactions of influencing parameters on COD

and lignin removal, as well as methane production rate can be evaluated. Thus, the main objective of this research is to explore the interaction and to investigate and optimize a novel MAHB reactor for COD and lignin removal and methane production rate using response surface methodology (RSM) and artificial neural network (ANN) by developing an active methanogens biomass in treating RPME.

MATERIAL AND METHODS

The schematic diagram of the laboratory-scale MAHB reactor is shown in Figure 1. The basic design of the MAHB reactor is rectangular in shape that contains five compartments which are separated by a modified vertical baffle. The reactor has a total effective volume of 58 L. Polypropylene ring packings are used as media for supporting the biofilm formation and these packing materials are located at the undersurface of compartments 2 and 3. Sampling ports are present on the top of each compartment and the reactors are maintained at a constant temperature of $35 \pm 0.2^\circ\text{C}$. Samples were collected from each compartment for analysis together with the effluent.

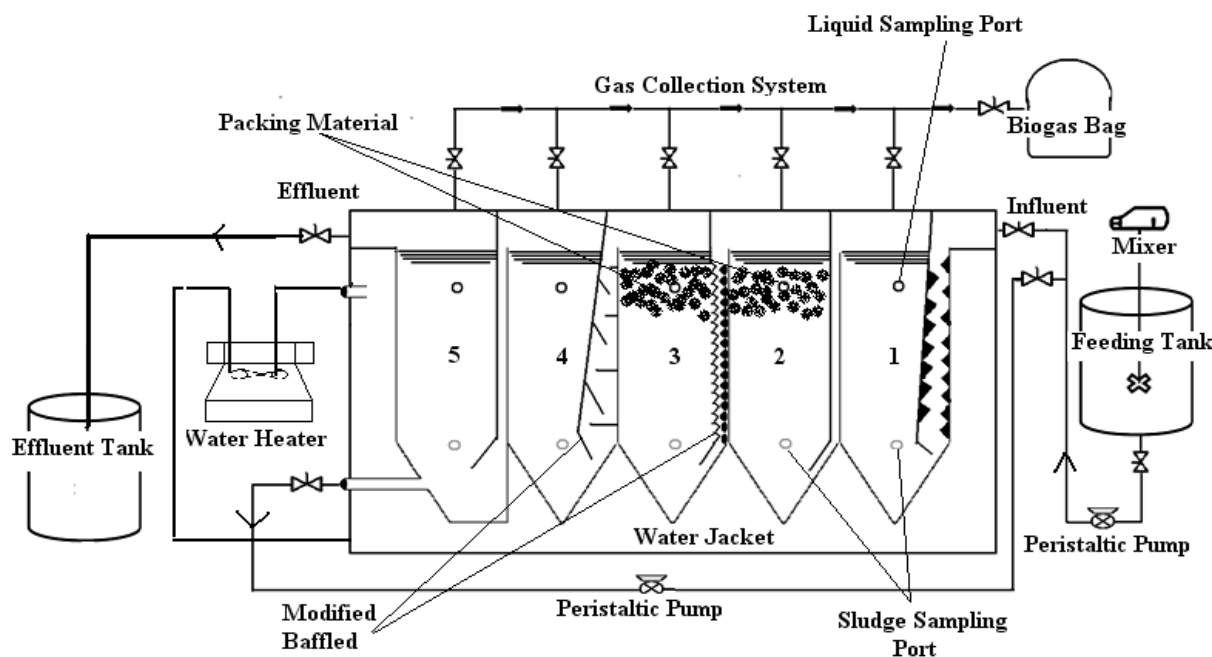


Figure 1. Schematic diagram of the modified anaerobic hybrid baffled reactor

Bioreactor operation

The inoculum used for seeding was anaerobic granular sludge (10% v/v) taken from the anaerobic pond of Malpom Industries Berhad, which was mixed with 750 mg L^{-1} COD of RPME taken from Muda Paper Mills Sdn Bhd. The characteristics of RPME used and the startup of MAHB reactor have been reported previously [11]. The steady-state

performance was evaluated at different influent COD concentrations ($1000\text{--}5000 \text{ mg L}^{-1}$) and hydraulic retention times (HRT) of 1-7 days. Variation of $\pm 5\%$ in effluent COD concentration at each condition was considered as the criterion for steady-state conditions. COD was measured using the spectrophotometer DR-2800 according to the reactor digestion method [12], while biogas composition was determined using Shimadzu GC-

FID with propack N column. Carrier gas was helium set at a flow rate of 50 ml min⁻¹, column temperature of 28°C, detector temperature of 38°C and injector temperature of 128°C. The lignin concentration was determined using DR-2800 spectrophotometer (HATCH model) by the tyrosine method. Triplicate samples were collected for each parameter reading to increase the precision of the results, and only the average value was reported throughout this study.

Experimental design and optimization

The statistical software Design-Expert 6.0 (Stat Ease Inc. Minneapolis, USA) was used to determine the optimal combination of parameters equivalent to the optimized responses achieved. D-optimal design of RSM was used in the optimization of COD and lignin removal and methane production rate from anaerobic digestion of RPME. The hydraulic retention time (HRT) and feeding COD concentration were chosen as the factors and the experimental ranges used are shown in Table 1. According to the design, a total of 12 runs of experiments were performed (Table 2). For optimal point prediction, experimental data were fitted with a second-order polynomial model. The adequacy of the model was evaluated by analysis of variance (ANOVA) and coefficient of determination, R². The model describes the interaction among the parameters influencing the response by varying them concurrently. For ANN, a narrower range of 3 – 5 days for HRTs and 2500 – 4500 mg L⁻¹ for feeding COD concentration was used. This ANN was done to investigate whether the data obtained fit well with the data from RSM and to achieve better values of performances in terms of responses.

Table 1. Experimental ranges and levels of the factors used

Factors	Range	
	-1	1
Hydraulic retention time/ HRT (days), x_1	1	7
Feeding COD concentration (mg L ⁻¹), x_2	1000	5000

Table 2. Factors used for interaction study by D-optimal design of RSM

Run	Hydraulic retention time/HRT (days),	Feeding COD concentration (mg L ⁻¹),
	x_1	x_2
1	4	1000
2	1	5000
3	1	5000
4	1	3000
5	1	1000
6	7	3000
7	4	3000
8	7	1000
9	7	5000
10	1	1000
11	4	5000
12	7	5000

All data used in RSM and ANN were taken during steady-state process for each experimental condition. Eighty one data points from 27 different continuous experiments were used as shown in Table 3. The 81 sample sets were split into training (80%), validation (10%) and testing (10%) data sets. The ranges for input and output data used to perform ANN optimization study are shown in Table 4.

A feed forward network with two hidden layers was used in this study which is trained using a backpropagation algorithm by means of Levenberg-Marquardt (LM) method. A two-layer ANN with sigmoidal transfer function was used for both hidden neurons and the input and output neurons.

RESULTS AND DISCUSSION

Response surface analysis regression

Two variables, namely hydraulic retention time (HRT) and feeding COD concentration were coded as x_1 and x_2 , respectively. The three responses - COD removal, lignin removal and methane production rate were denoted as Y_1 , Y_2 and Y_3 , respectively. The responses in coded terms are summarized in Table 5.

Table 3. Experimental conditions

Type of wastewater	No of batches	No of data points	HRT (days)	Influent COD (mg L ⁻¹)
RPME	27	81	3 -5	2500 – 4500

Table 4. Range of input and output parameters used in ANN

Parameters	Minimum	Maximum	Units
HRT	3	5	days
Influent COD	2500	4500	mg L ⁻¹
Methane production rate	0	8.09	L CH ₄ day ⁻¹
COD removal	0	97	%
Lignin removal	0	60	%

Table 5. Results of response surface design analysis

Run	Variables		Responses			
	x_1 (day)	x_2 (mg L ⁻¹)	Y_1 (%)	Y_2 (%)	Y_3 (L CH ₄ day ⁻¹)	Y_3 (L CH ₄ gCOD ⁻¹ day ⁻¹)
1	4	1000	95.41	51.16	4.903	4.90
2	1	5000	94.28	43.13	4.354	0.87
3	1	5000	94.45	45.81	4.116	0.82
4	1	3000	95.45	49.47	7.362	2.45
5	1	1000	94.20	42.67	5.317	5.32
6	7	3000	95.86	49.62	6.817	15.85
7	4	3000	97.93	62.83	8.475	11.30
8	7	1000	94.29	45.00	3.403	3.40
9	7	5000	94.41	40.54	5.097	7.18
10	1	1000	94.46	42.67	5.646	5.65
11	4	5000	95.59	49.23	5.231	4.18
12	7	5000	94.18	45.87	5.197	7.32

As can be seen, Y_1 gives values of ≥ 92.18 %, Y_2 gives a value of ≥ 40.54 % and Y_3 gives a value of ≥ 3.403 L CH₄ day⁻¹. All responses were then analysed to investigate the interaction and to predict the optimum values. The experimental data were analysed and results indicated that the models are greatly significant with very low probability values (<0.0001 - <0.0074) which signifies that independent variables model terms were significant at 95% confidence level.

(a) *Effect of independent variables on COD removal.* COD removal efficiency was found to be a function of hydraulic retention time, (HRT) and feeding COD concentration. The feeding COD concentration affects the removal efficiencies since the substrate-to-microorganism ratio is affected by the feeding COD concentration. The experimental results show that COD removals are between 94 - 98 % in the range of COD concentrations and HRT studied. These results indicate that the MAHB reactor had a higher ability to resist shocking load at high COD concentrations. The compartmentalization of the MAHB reactor offered good phase separation that is able to convert substrate in depth, which acts as a main contributor

to the high COD removal in this system. Multiple regression analysis was applied on the experimental data and the results are fitted with second order polynomial equation. The relationship between COD removal and the two variables in coded terms is shown in Equation (1):

$$Y_1 = 97.42 + 0.013x_1 + 0.032x_2 - 1.51x_1^2 - 1.66x_2^2 - 1.934E-004x_1x_2 \quad (1)$$

where Y_1 is COD removal (response) in percentage, x_1 and x_2 are coded terms for feeding COD concentration (mg L⁻¹) and hydraulic retention time (days), respectively. The results using analysis of variance (ANOVA) for COD removal responses are as tabulated in Table 6. The *F-values* and *p-values* are used to measure the degree of significance of each coefficient. Larger *F-values* and smaller *p-values* denote higher significance of the corresponding coefficients. *P-values* less than 0.0500 indicate significant model terms. In this case, the square effects of HRT (x_1^2) and feeding COD concentration (x_2^2) were significant in terms of the model. The model *F-values* of 20.52 and *p-values* less than 0.0010 indicate significant model terms.

Table 6. ANOVA results for COD removal responses

Model term	Coefficient estimate	Standard error	F-value	p-value
Intercept	97.42	0.26	20.52	0.0010
x_i	0.013	0.12	0.012	0.9165
x_2	0.031	0.12	0.071	0.7984
x_1^2	-1.51	0.24	40.80	0.0007
x_2^2	-1.66	0.24	49.53	0.0004
x_1x_2	-0.0001934	0.14	0.000001993	0.9989

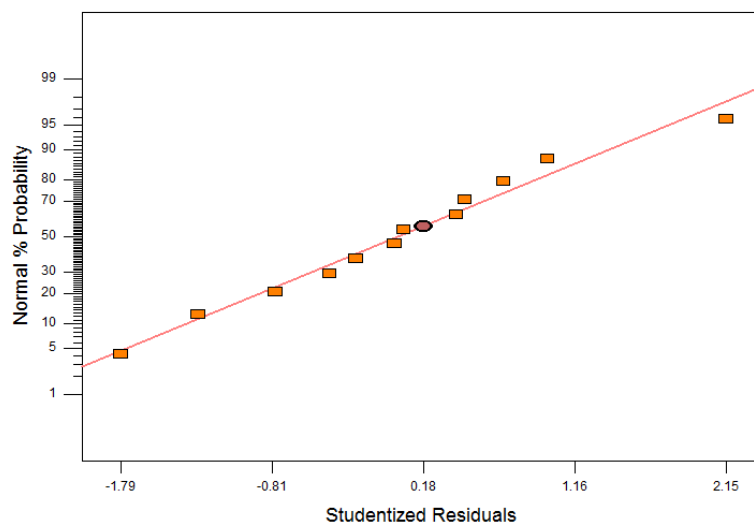


Figure 2. COD removal plot of normal probability with respect to HRT and feeding COD.

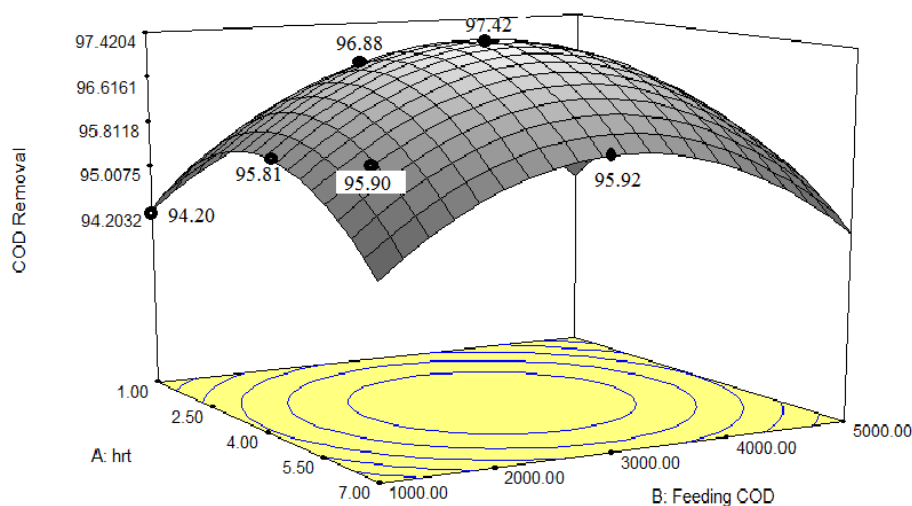


Figure 3. COD removal 3D plots predicted with respect to HRT and feeding COD.

Figure 2 shows the normal probability plot of residuals for COD removal which can be used to indicate whether the standard deviation of actual and predicted responses follows the normal distribution. The figure shows that all residuals are near to a straight line and no severe sign of abnormality of the experimental data is denoted.

This suggests that the underlying error is normally distributed. A portrayal of the 3D plot of COD removal with respect to the two variables is shown in Figure 3. Results indicate that the two variables influence the response as defined earlier.

As shown in Figure 3, as the variables (HRT and feeding COD concentration) increase, an increase

in the response is achieved. Then, as the optimum condition is achieved (highest peak of removal efficiency), a reduction in responses is noted once the variables are further increased. Based on Figure 3, an increase in HRT from 1 to 4 days and in feeding COD concentration from 1000 to 3000 mg L⁻¹ caused a remarkably high COD removal, (95 – 96%). The interaction of variables towards responses can be explained through the contour plot. Variable x_1 is fixed at 4 days to analyse the effect of increasing x_2 towards Y_1 . As can be seen, as the values of x_2 increase from 1000 to 2000 mg L⁻¹, the response Y_1 shows an increment from 95.81 % to 96.88 %. Further increase of x_2 gives an optimum value of Y_1 of 97.42 %. However, as x_1 further increases to 5000 mg L⁻¹, a slight reduction of Y_1 is noticed. The reduction of responses at higher x_2 was mainly attributed to the greater toxicity to methanogens in the long run. This is due to the low biodegradability of the microorganisms that cause acid accumulation, which affects the activity of some bacteria and contributes to deteriorate the effect on the reactor performance [13]. For a fixed variable x_2 at 3000 mg L⁻¹, an increment of x_1 from 1 to 4 days shows an increment in Y_1 from 95.90 to 97.42 %. However, further increments of x_1 to 7 days show a reduction in value of Y_1 of 95.92 %. From the interaction of contour plots, it is clearly seen that the maximum value of the predicted COD removal efficiency is 97.42 % at HRT of 4 days and feeding COD concentration of 3000 mg L⁻¹, whereas the minimum predicted response (95.73%) was achieved at HRT of 4 days and feeding COD of 1000 mg L⁻¹.

(b) *Effect of independent variables on lignin removal.* ANOVA results for lignin removal are as shown in Table 7.

Table 7. Regression analysis using D-optimal response surface methodology

Model term	Coefficient estimate	Standard error	F-Value	p-Value
Intercept	59.6	1.93		
x_i	0.18	0.91	0.04	0.8463

(c) *Effect of independent variables on methane production rate.* One important factor that indicates better microbial activities inside the MAHB reactor is the production of biogas, specifically methane gas. High methane production indicates that the methanogenic bacteria are in good activity and consume the substrate inside to produce methane.

x_2	-0.15	0.91	0.026	0.8772
x_1^2	-8.43	1.78	22.41	0.0032
x_2^2	-7.79	1.78	19.11	0.0047
x_1x_2	-0.84	1.03	0.67	0.4458

From the results, the square effect, x_1^2 and x_2^2 shows significant model terms of the response. Linear effect (x_1 and x_2) and two level interaction (x_1x_2) have probability values > 0.05 which are not significant. This is because the extracellular enzymes required for de-polymerization of lignin need molecular oxygen, and their oxidative reactions would not be anticipated under anaerobic conditions. However, results indicate that both variables do not contribute to the production of the particular extracellular enzymes. The model for coded factors of lignin removal is shown in Equation (2):

$$Y_2 = 59.60 + 0.18x_1 - 0.15x_2 - 8.43x_1^2 - 7.79x_2^2 - 0.84x_1x_2 \quad (2)$$

Figure 4 shows a contour plot with respect to the variables. From the contour plots, it can be seen that the response increases as the HRT is close to 4 days and feeding COD is 3000 mg L⁻¹.

For the fixed variable x_2 at 3000 mg L⁻¹, an increment of x_1 from 1 to 4 days shows an increase in Y_2 from 50.98 to 59.60 %. However, further increments of x_1 to 7 days show a reduction in the value of Y_1 of 51.35 %. The interaction of variables towards responses can be explained through the contour plot. Once the variable x_1 is fixed at 4 days and x_2 increases from 1000 to 3000 mg L⁻¹, the response Y_2 shows an increment from 51.96 to 59.60 %. With further increase of x_2 to 5000 mg L⁻¹, a slight reduction of Y_2 is noticed. From the interaction of contour plots, it is clearly seen that the maximum value of the predicted lignin removal efficiency is 59.60 % at HRT of 4 days and feeding COD concentration of 3000 mg L⁻¹, whereas the minimum predicted response (42.49 %) was achieved at HRT of 1 day and feeding COD of 1000 mg L⁻¹. This indicates that intermediate values of both HRT and feeding COD concentration yield a good performance in removing lignin.

The results for ANOVA of methane production are shown in Table 8.

P-value greater than 0.0500 indicates that the model terms are not significant. From the analysis, x_1 , x_1^2 , x_2^2 and x_1x_2 are significant model terms. The coded factors model for lignin removal is shown in Equation (3):

$$Y_3 = 8.06 - 0.30x_1 + 0.14x_2 - 0.77x_1^2 - 2.79x_2^2 + 0.76x_1x_2 \quad (3)$$

Figure 5 shows the predicted versus actual plot for methane production with R² value of 0.9798 which demonstrates a reasonable degree of correlation between experimental and predicted

values. The contour plots of the quadratic model for methane production with respect to HRT (x₁) and feeding COD (x₂), within the space design are shown in Figure 6.

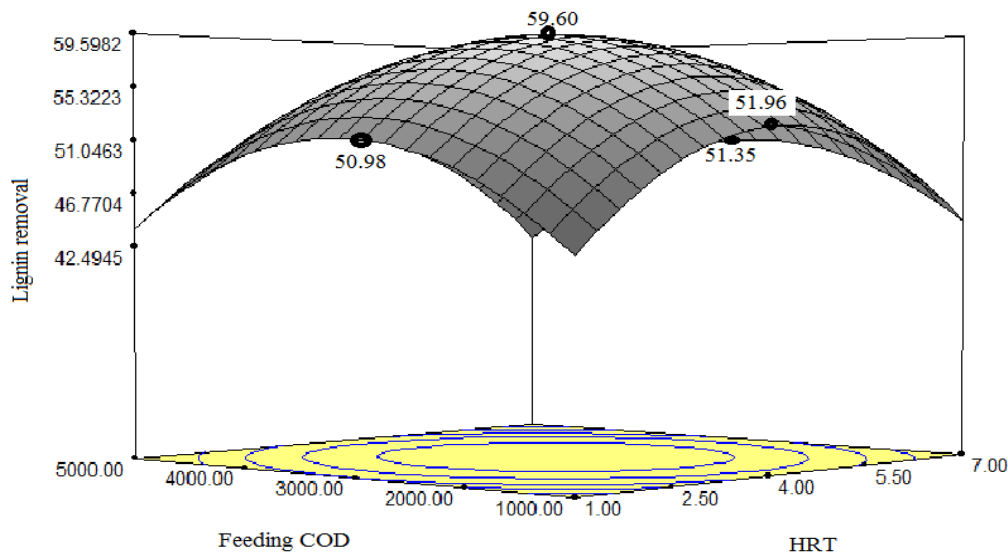


Figure 4. 3D plot with respect to HRT and feeding COD.

Table 8. Regression analysis using D-optimal response surface methodology

Model term	Coefficient estimate	Standard error	F-Value	p-Value
Intercept	8.06	0.20	58.10	0.0001
x ₁	-0.30	0.09	9.950	0.0197
x ₂	0.14	0.09	2.270	0.1823
x ₁ ²	-0.77	0.19	17.23	0.0060
x ₂ ²	-2.79	0.19	226.55	0.0001
x ₁ x ₂	0.76	0.11	49.97	0.0004

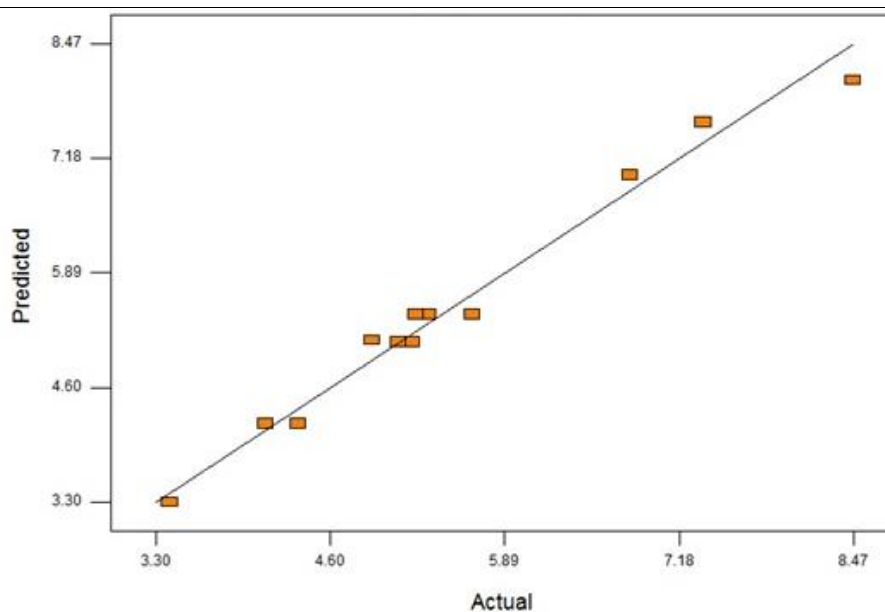


Figure 5. Predicted vs actual values plot for methane production rate.

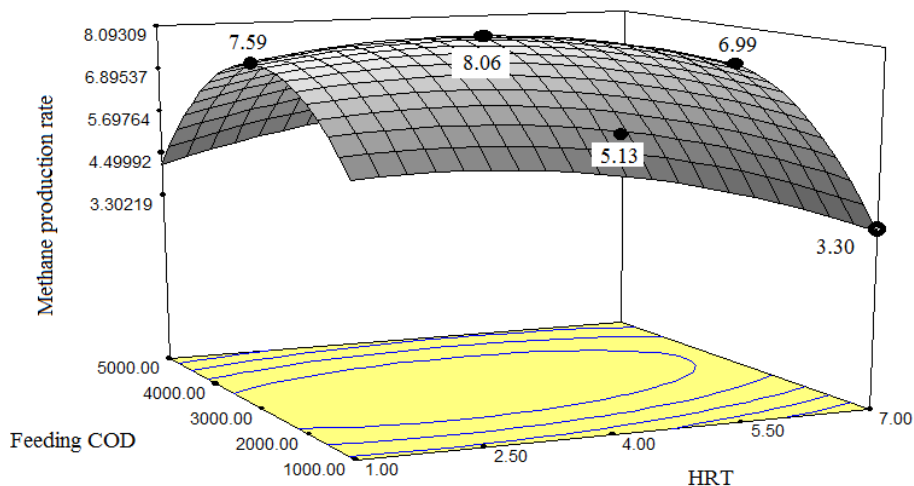


Figure 6. Response surface and counter plots for methane production rate with respect to HRT and feeding COD.

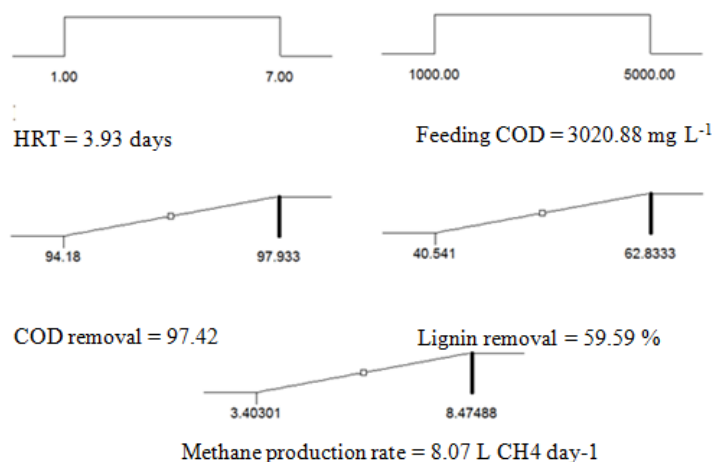


Figure 7. Desirability ramp for numerical optimization of four goals, namely the initial solution pH, initial lead ion concentration, biomass dosage and lead removal.

As can be seen, at fixed variable x_2 at 3000 mg L⁻¹ and increment of x_1 from 1 to 4 days there is an increase in Y_3 from 7.59 to 8.06 L CH₄ day⁻¹. However, a further increment of x_1 to 7 days shows a reduction in the value of Y_1 to 6.997 L CH₄ day⁻¹. For the variable x_1 , once the variable is fixed at 4 days and x_2 is increased from 1000 to 3000 mg L⁻¹, the response Y_3 shows an increment from 5.13 to 8.06 L CH₄ day⁻¹. Further increase of x_2 to 5000 mg L⁻¹ shows a slight reduction of Y_3 . From the interaction of contour plots, it is clearly seen that the maximum value of the predicted methane production rate is 8.06 L CH₄ day⁻¹ at HRT of 4 days and feeding COD concentration of 3000 mg L⁻¹, whereas the minimum predicted response (3.30 L CH₄ day⁻¹) is achieved at HRT of 7 days and feeding COD of 1000 mg L⁻¹. Intermediate feed strength (3000 mg L⁻¹) and HRT (4 days) gave an optimum condition in terms of organic load and feed flow rate that permitted the biomass or

microbes inside to become contact to the substrate to digest it and produce methane. The interaction indicated that HRT and feeding COD concentration played a significant role in methane production in the reactor.

(d) *Optimization using desirability function.* There are various options for the data that can be set during this optimization including minimizing, maximizing, setting to exact values or setting the data within the range of study. The shape of particular desirability function was adjusted by assigned weight to each goal, then combined into an overall desirability function. Figure 7 shows the desirability ramp for optimization of five goals, namely HRT, feeding COD concentration, COD removal, lignin removal and methane production rate. The maximum desirability function was set within a range of HRT from 1 to 7 days, feeding COD concentration from 1000 to 5000 mg L⁻¹, and maximize level of COD removal efficiency (97.93%), lignin removal efficiency (62.8%) and

methane production rate ($8.47 \text{ L CH}_4 \text{ day}^{-1}$). The best local maximum found was at HRT of 3.93 days, feeding COD concentration of $3020.88 \text{ mg L}^{-1}$, COD removal efficiency of 97.42 %, lignin removal efficiency of 59.59 % and methane production rate of $8.07 \text{ L CH}_4 \text{ day}^{-1}$ with desirability value of 0.897. The obtained desirability value indicates that the estimated function signifies the desired conditions and experimental model. Interaction of variables towards MAHB reactor performance in terms of COD removal, lignin removal and methane production rate was successfully investigated. From the RSM result, ANN analysis was then used to compare the results (analysis) using ANN in order to check whether the data fit well with the actual data and predict the optimum performance of the MAHB reactor in treating RPME. The results are further discussed in the following section.

Analysis of COD and lignin removal and methane production using neural network

In the present study, the experimental data were compared with the predicted optimum data obtained from RSM analysis to evaluate the ANN ability. The experimental data were chosen from the RSM analysis. Three different data set were used for fitting purpose which are 54 data for training process, 81 data for validation and 36 data for testing. Mean square error (MSE) and regression R^2 values were used to evaluate the performance and validation of ANN. Figure 8 shows the regression plot of neural network generated by ANN toolbox. As can be seen, the R values for all training, validation and testing sets were 0.99996, 0.99989 and 0.9998, respectively. This indicates that the ANN gave good agreement between the outputs and predicted values. The correlation between outputs and predicted values indicates that higher R values represent closer interaction while zero R

indicates random interaction. In training process, the weights of the ANN were adjusted in order to minimize the values of MSE. From the regression plots (Figure 8), it can be seen that R values were closer to 1 which suggests that the predicted values from the ANN analysis have a linear correlation with experimental data. Hence, it can be concluded that the ANN toolbox is a good indicator in predicting the outcome of the COD removal efficiency, methane production rate and lignin removal efficiency from the MAHB reactor.

Figure 9 shows the MSE *versus* epochs for training, validation and testing. The ANN fitting revealed that the smallest MSE obtained was 0.34249 at an epoch of 7. The correlations between experimental data and output predicted by ANN using ANN toolbox are shown in Figure 10. As can be seen, a good agreement between experimental and ANN outputs was achieved for all outputs of COD removal, lignin removal and methane production rate with R^2 values of 0.970, 0.9906 and 0.9545, respectively. Table 9 summarizes the optimum predicted COD removal efficiency, lignin removal efficiency and methane production rate using RSM and ANN toolbox.

Results show that predicted COD removal efficiency are 97.42 and 98.16 %, lignin removal efficiency - 59.59 and 77.29 % and methane production rate - 8.07 and 8.34 L day^{-1} for RSM and ANN, respectively. The predicted values for COD removal, lignin removal and methane production for both RSM and ANN are most likely to be similar to the actual values. This also indicates that the validation and fitting using ANN toolbox yield close optimum predicted results as compared to the predicted results previously obtained using RSM.

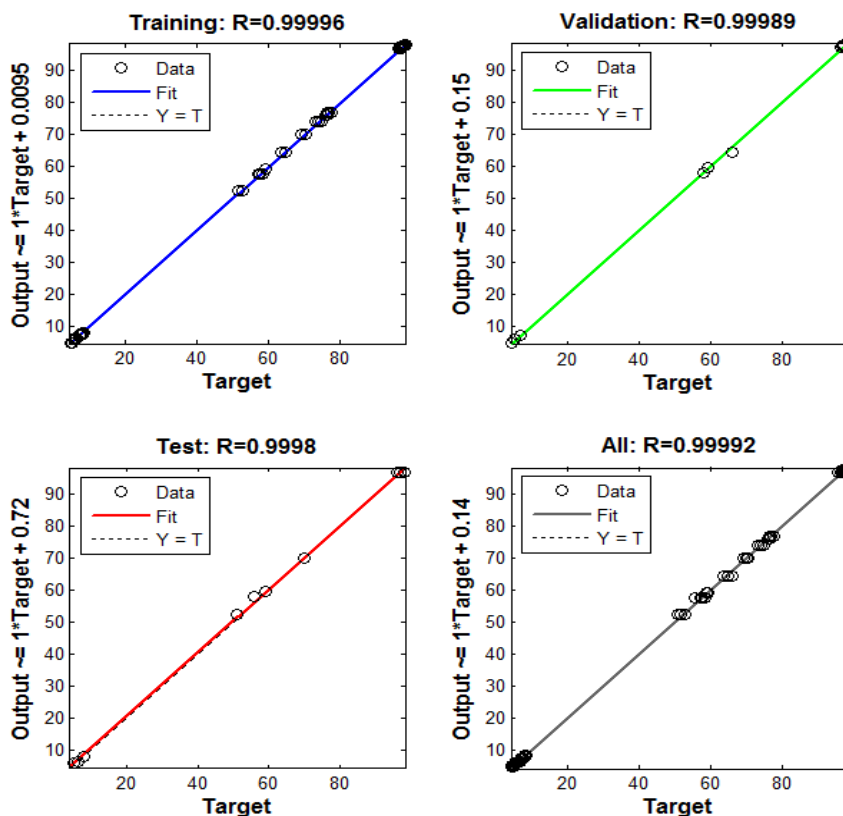


Figure 8. Regression plots of the neural network model.

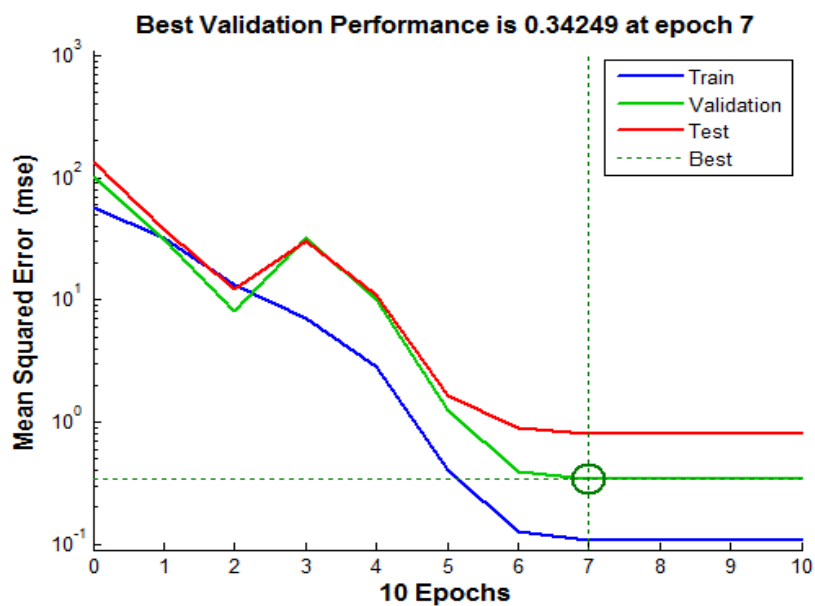


Figure 9. Training, validation, and test square mean errors for Levenberg-Marquardt algorithm

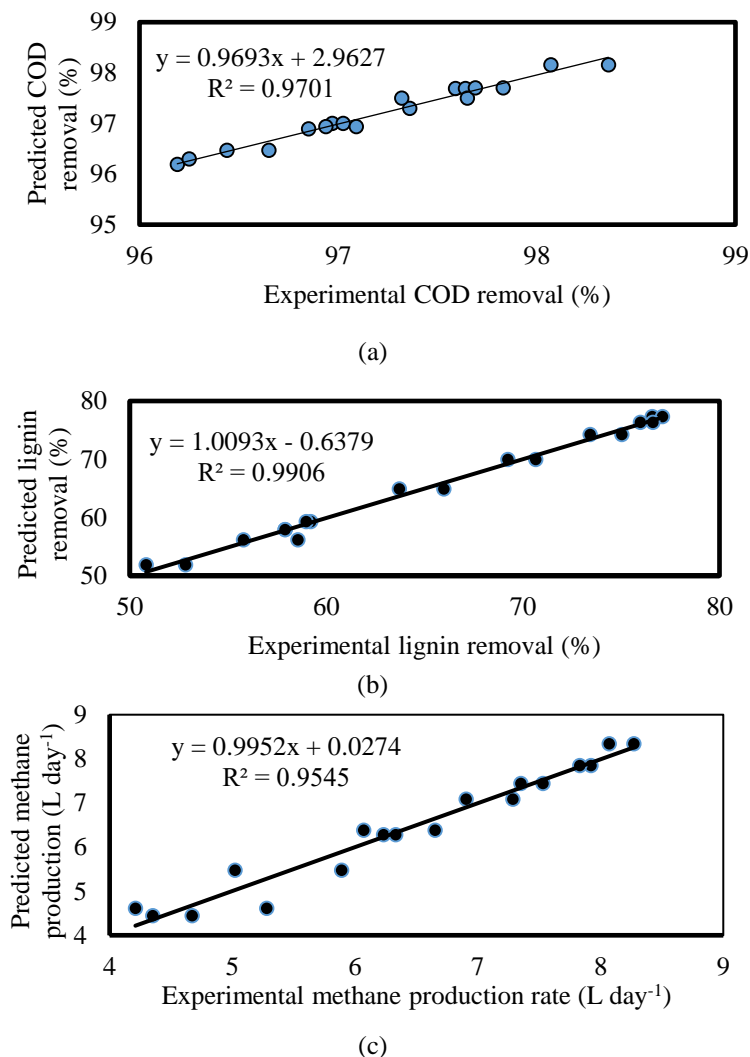


Figure 10. Correlation between experimental and predicted ANN data for (a) COD removal efficiency, (b) lignin removal efficiency and (c) methane production rate

Table 9. Optimum predicted output obtained by RSM and ANN

	COD removal	Lignin removal	Methane production rate (L day ⁻¹)
RSM	97.42	59.59	8.07
ANN	98.16	77.29	8.34
R ²	0.970	0.990	0.954

CONCLUSIONS

The HRT and feeding COD concentration variables showed significant effects towards the increase of responses. Intermediate HRT and COD concentrations significantly enhanced the COD and lignin removal and also gave the highest methane production rate. The predicted COD removal efficiency was 97.42 and 98.16 %, lignin removal was 59.59 and 77.29 % and methane production rate was 8.07 and 77.29 L day⁻¹ for RSM and ANN, respectively, which indicates that the predicted values for both methods are most likely to be

similar to the actual values. This designates that the validation and fitting using ANN toolbox yield close optimum predicted results to those predicted by RSM. Higher R² values achieved demonstrated that both methods could be efficiently used for the prediction of COD and lignin removal and methane production by anaerobic digestion of RPME using MAHB reactor.

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