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Optimization of Methane Production from Macroalgae Feedstock using Multivariate Technique under Mesophilic and Thermophilic Conditions

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Abstract

A multivariate technique was used to optimize methane production from anaerobic digestion of macroalgae under mesophilic and thermophilic conditions. To evaluate the effects and interaction of three reaction variables: COD, VFA, and ammonia on methane production, their data recorded in a time order were subjected to fit and multiple regression analysis, which generated a second order quadratic polynomial equation used to predict the optimized methane production. The ANOVA results showed the developed model for the mesophilic ($p< 0.003$) and thermophilic ($p< 0.000$) reactors are significant. Their R^2 values of 0.97 and 0.99 suggest it was suitable for interpreting the experimental data set and adjusted R^2 of (0.91 and 0.97) indicates good regression models. The interaction terms $\boldsymbol{X_2^2}$ $\boldsymbol{(vfas)}$ $\boldsymbol{and}$ $\boldsymbol{X_1 \times X_2}$ $\boldsymbol{(COD, VFAs)}$ for mesophilic and thermophilic reactors, has a positive influence on methane production compared to other terms. The model predicted the optimal reactors conditions, derived as X_1: COD = 6.6 g L^{-1}, X_2: VFAs = 2.8 g L^{-1}, X_3: Ammonia = 1.3 g L^{-1} for the mesophilic reactor, and X_1: COD = 6.7 g L^{-1}, X_2: VFAs = 2.5 g L^{-1}, X_3: Ammonia = 1.1 g L^{-1} for the thermophilic reactor.

Keywords: Biomethane; macroalgae; regression; optimization; quadratic.

1. Introduction

Since the first use of anaerobic digestion technology to generate biogas to power street lights in Britain, significant advances have been developed to optimize the process in a sustainable manner [1].

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The multi-objective of optimization of biomass feedstocks to biogas is to enhance material and energy efficiency with reduction in emission [2]. Over the years extensive studies has been carried out on biogas optimizing and better substrate evaluation [3]. In optimizing anerobic digesters for increased biogas yield, certain key variables as pH, volatile fatty acids (VFAs), alkalinity, suitable organic loading rate, temperature and carbon to nitrogen ratio (C:N) among others variables has been suggested as critical monitored parameters, and also an appropriately designed anaerobic digester is central for the production of optimized biogas [1][4]. The world attention has been drawn to algae as a novel biomass source for renewable energy [5]. Algae feedstocks are regarded as a useful underestimated resources for biobased economy because their cells contain a range of beneficial compounds with high biological activity [6], and macroalgae, in particular, have the potential of becoming viable aquatic energy crop but energy production from macroalgae is still limited due to economic viability [7]. Current biofuels from algae is illustrated in Figure 1. Various traditional fermentation optimization techniques or models has been used to increase biogas production [8] and particularly the surface response methodology (RSM) for optimal biogas production from macrolagae [9]. This study looked at optimization of the biogas production potential from macroalgae, using a multivariate technique and surface and contour plots analysis to predict parameters interaction effects for optimal conditions.

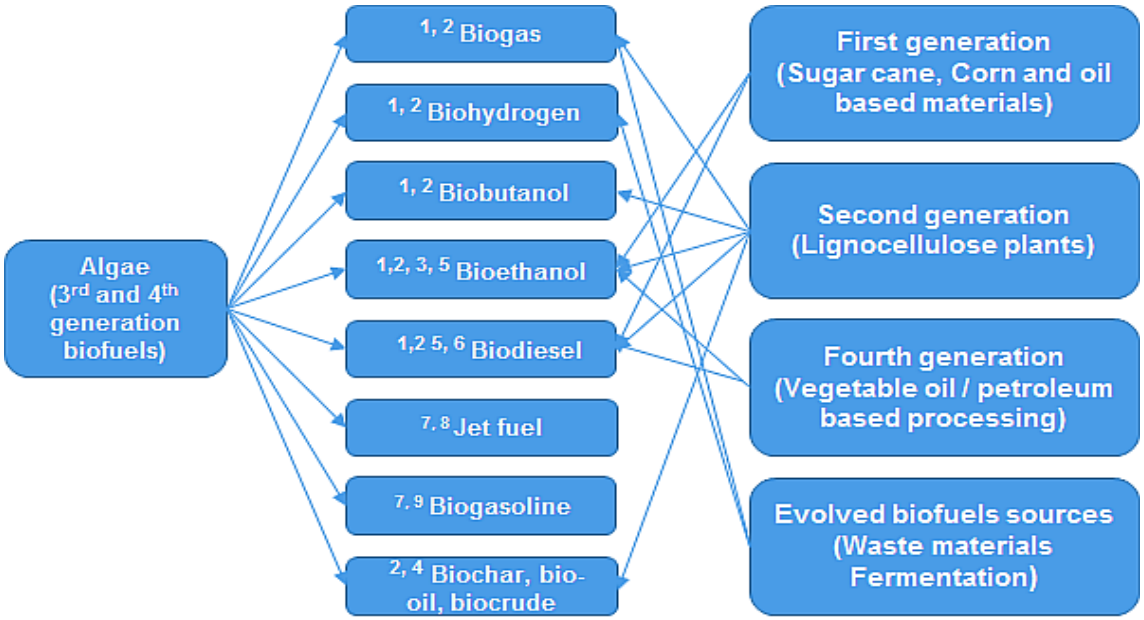

Figure 1: Renewable fuel sources and bioproducts from algae [10]

2. Materials and methods

2.1. Algae collection, pretreatment, and storage

Algal biomass Laminaria digitata (LD) used in the continuous reactor experiments were collected from shallow water during low tide at Culler coats Bay, 55.0342° N, 1.4309° W, Tyne and Wear (NZ3572), United Kingdoom in December 2017. The seaweeds were transported in 30 liter bags and were immediately washed to remove marine salts and sediments. Only the fronds were used and was roughly chopped by hand to particle size of about 10 mm using knife, approximately 250 g were then macerated and oven dried at 70 °C for 24 - 48 hrs.

Dried samples were then pulverized with a Kenwood 100 coffee blender to particle size generally < 1mm. All samples were stored at 4 °C in an airtight gas bag until required.

2.2. Analytical methods

2.2.1. Chemical oxygen demand (COD)

Chemical oxygen demand (COD) analysis was carried out using commercially available COD kits (Merck, UK). Diluted sample were centrifuged at 3600g for five minutes, and supernatant were then filtered through a 0.20 µm syringe filter (VWR, UK). 3ml of this filtered sample was added to COD tubes and digested at 150 °C for 2 hrs. The COD values were determined by spectroscopic absorbance using a Spectroquant Nova 60 (VWR, UK) colorimeter [11].

2.2.2. Ammonical nitrogen (NH_3 -N)

Ammonical nitrogen (NH_3 -N) was determined using a Vapodest 30S steam distillation apparatus (C Gerhardt Lab Supplies, UK). Fifty milliliters of sample were placed in a Kjeldahl digestion tube, with few drops of phenolphthalein indicator and adjusted to pH above 8.3 using NaOH where necessary. Borate buffer solution (3 ml) was added to the mixture and distilled into 50 ml of boric acid indicator. The distillate was titrated with 0.02 N H_2SO_4 to a pale lavender endpoint. A reagent blank was distilled and titrated in the same way and subtracted from the sample titer to calculate the NH_3–N of the sample [11].

2.2.3. Volatile fatty acids (VFAs)

Volatile fatty acids (VFAs) was analyzed on a Dionex ICS 1000 with an AS40 autosampler (Dionex, USA). Separation was carried out on an ionpac ICE-AS1 4 × 250 mm analytical column with a flow rate 16 ml min^{-1}; 1.0mM heptafluorobutyric acid eluent; 5 mM tetrabutylammonium hydroxide suppressant regenerant; and a 10ul injection loop. Supernatant of centrifuged samples liquors were filtered through a 0.20 µl syringe filter (VWR, UK), 0.4 ml of filtered samples were then diluted 1:1 with octane sulfonic acid, and sonicated (FS200B Sonic Bath, Decon Laboratories, Sussex, UK) for 40 mins to remove carbonate, which caused interference. The prepared samples were then transferred to 1 ml tubes with filter caps (Dionex, USA) before analysis.

3. Experimental procedure

The continuous reactor comprised of 2 identical, 1-litre continuous stirred tank reactors (CSTR) operating simultaneously for 127 days with the same daily feeding regime (seaweed feedstock) under mesophilic 35 °C (MR 1) and thermophilic 55 °C (TR 1) conditions, with a hydraulic residence time of 25 days. The initial inoculum concentration was 10 g VS L^{-1} and the organic loading rate (g VS L^{-1} d^{-1}) was increased stepwise after acclimatization from 1 g VS L^{-1} d^{-1} on day 1 of the experiment to 2 g VS L^{-1} d^{-1} on day 15, thereafter, to 3 g VS L^{-1} d^{-1} on day 70, 4 g VS L^{-1} d^{-1} on day 90 and, finally to 5 g VS L^{-1} d^{-1} on day 98, till the end of the experiment in both temperature conditions. Biogas production rate was measured daily for the first 40 days, after which it was measured every 2 days according to methods reported elsewhere [12].

3.1.1. Optimisation methodology used

The optimization process employed an approach using fit and multiple regression analysis by exploring the relationships between experimentally determined time series data set, as *continuous predictors* variables (independent), and an output, as a *response variable* (dependent), methane produced. The fit regression model was used to fit the data set (response against predictors variables) to generate an ANOVA equations and interactions terms while the multiple regression model was used to optimize methane production by evaluating the influence and interactive effects of the data set (predictor variables). The model employed, use the fit and multiple regression analysis tool in Minitab 17, to obtain the interactions between experimentally determined methane production and observed process parameters. The coefficient of determination (R^2) value obtained expresses the adequacy and quality of the model fitness and the interactions terms were evaluated by a p-value of 95%($p>0.05$). The experimental data results (pH, COD, VFA, ammonia, and alkalinity) for both the mesophilic (MR 1) and thermophilic (TR 1) reactors were subjected to correction test using matrix plot to check for correlation among the variables known as multiple collinearities, which can cause instability in the model [13]. The elimination method was then applied to remove correlated parameters using ($p <0.05$) both for the mesophilic reactor (MR 1) and thermophilic reactor (TR 1). The parameters COD, VFA and ammonia were then selected as adequate from the outcome of the correlation results to fit the model [13]. The selected parameters, their data set which were recorded in a time order, data for the continuous digestion process for COD (Figure 2), VFA (Figure 3), and ammonia (Figure 4) were applied as continuous predictors variables, and fitted against the methane production values (Figure 5) for reactors MR 1 and TR 1, Table 1. These were then used to generate an ANOVA quadratic equation. The interactions terms in the equation were then used to describe and predict the optimised methane production from optimal predicted conditions of the reactors (MR 1 and TR 1). Multiple regression has been previously used by several authors in various studies for methane optimization and for optimization of anaerobic digestion of macroalgae [14,15].

Table 1: Variables used in fit and multiple regression analysis.

Factor	Levels	Response (Reactors MR 1 and TR 1)
Seaweed specie *(L. digitata)*	COD VFA Ammonia	Methane production (mL / gVS_{added}.reactor)

4. Results and Discussion

4.1. Model equation generation: Mesophilic temperature

For the mesophilic reactor MR 1, the result of the quadratic second –order multiple regression in form of ANOVA is shown in Table 2.

Table 2: Analysis of Variance (ANOVA) for Mesophilic reactor MR 1

Source	DF	Adj SS	Adj MS	F-Value	P-Value	Rank
Model	9	613793608	68199290	17.77	0.003	Significant
COD	1	27945565	27945565	7.28	0.043	Significant
VFA	1	8020199	8020199	2.09	0.208	5
Ammonia	1	17150329	17150329	4.47	0.088	4
COD*COD	1	19288051	19288051	5.03	0.075	3
VFA*VFA	1	3887407	3887407	1.01	0.360	7
Ammonia*Ammonia	1	2467699	2467699	0.64	0.459	9
COD*VFA	1	2503022	2503022	0.65	0.456	8
COD*Ammonia	1	22705161	22705161	5.92	0.059	2
VFA*Ammonia	1	4071449	4071449	1.06	0.350	6
Error	5	19190894	3838179			
Total	14	632984502				

$R^2 = 0.9697$; Adj.$R^2 = 0.9151$

The following quadratic equation and 2-way interactions terms was generated, Equation 1.

CH_4 production (Y) Equation 1

$$= -96148 + 20.71\,X_1 + 6.44\,X_2 + 49.7\,X_3 - 0.000835\,X_1^2 + 0.000497\,X_2^2 - 0.00420\,X_3^2 - 0.000460\,X_1 \times X_2 - 0.00641\,X_1 \times X_3 - 0.00268\,X_2 \times X_3$$

Where X_1: COD, X_2: VFA, X_3: ammonia.

Equation 1, shows the methane production as predicted (Y), as a function of the observed experimental process parameters (X_1, X_2, X_3,).

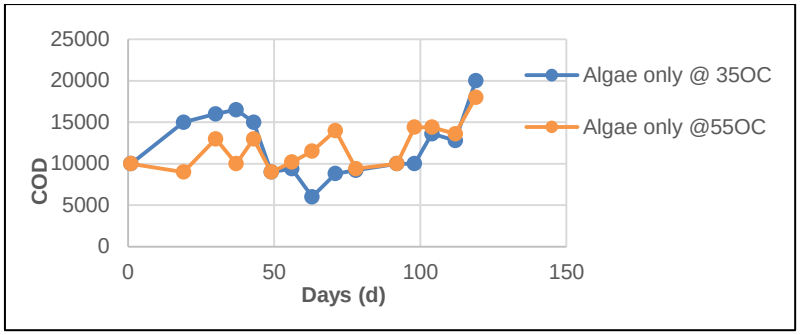

Figure 2: Chemical Oxygen Demand (COD) production for MR 1 and TR 1

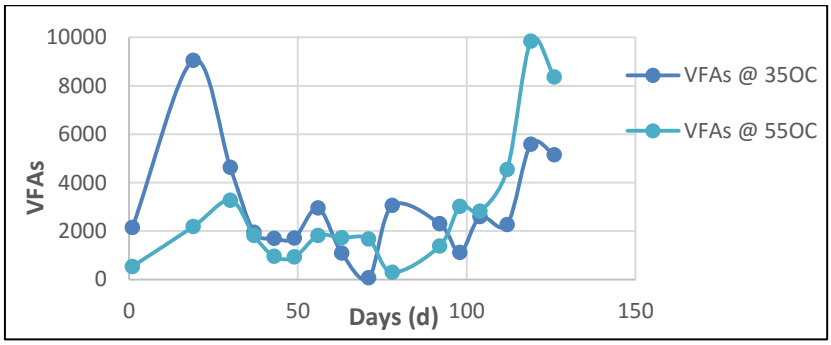

Figure 3: Volatile fatty acids (VFAs) production for MR 1 and TR 1

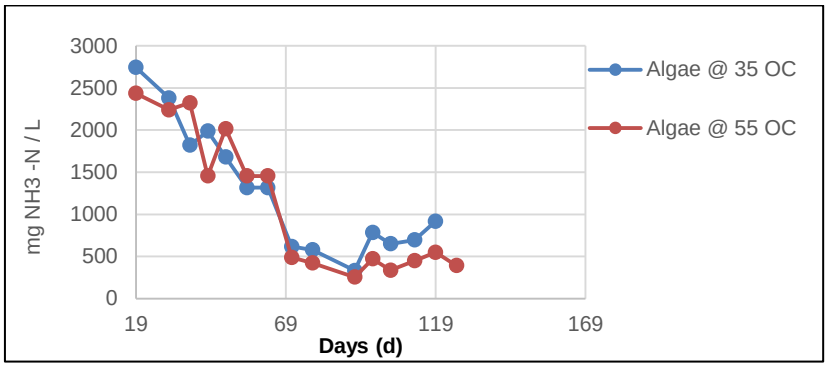

Figure 4: Ammonia production for MR 1 and TR 1

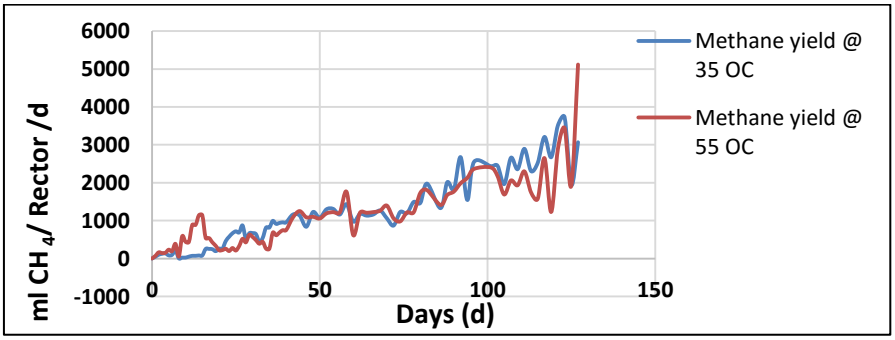

Figure 5: Methane yield MR 1 and TR 1

The relationship between Y and the X variables in the model is statistically significant with a $p < 0.003$ (Table 2). The R^2 value of 0.97 suggests it was appropriate for simulating the experimental data set [16]. Since, the goal is to maximize CH_4 production, using the model as a predictive tool, solution to optimal conditions obtained from the model building sequence of the interactive terms using multiple regression is; X_1: COD = 6.6 mg L^{-1}, X_2: VFAs = 2.8 g L^{-1}, X_3: Ammonia = 1.3 g L^{-1}. Using the coefficients in Equation 1 [17], and the ranking in Table 2, the predicted impacts of the variables on methane production is: $X_1 > (X_1*X_3) > (X_1*X_1) > X_3 > X_2 > (X_2*X_3) > (X_2*X_2) > (X_1*X_2) > (X_2*X_2)$, with X_1 (COD) concentration having the most impacts, followed by the interactions of COD and ammonia concentration (X_1*X_3), with ammonia interactions (X_2*X_2), being the least. The impact between COD and VFAs (X_1*X_2), and ammonia interactions $(X_2\text{^}2)$ are of the same magnitude. The main effects and interactions among the various variables from multiple regression analysis are shown in Figure 6.

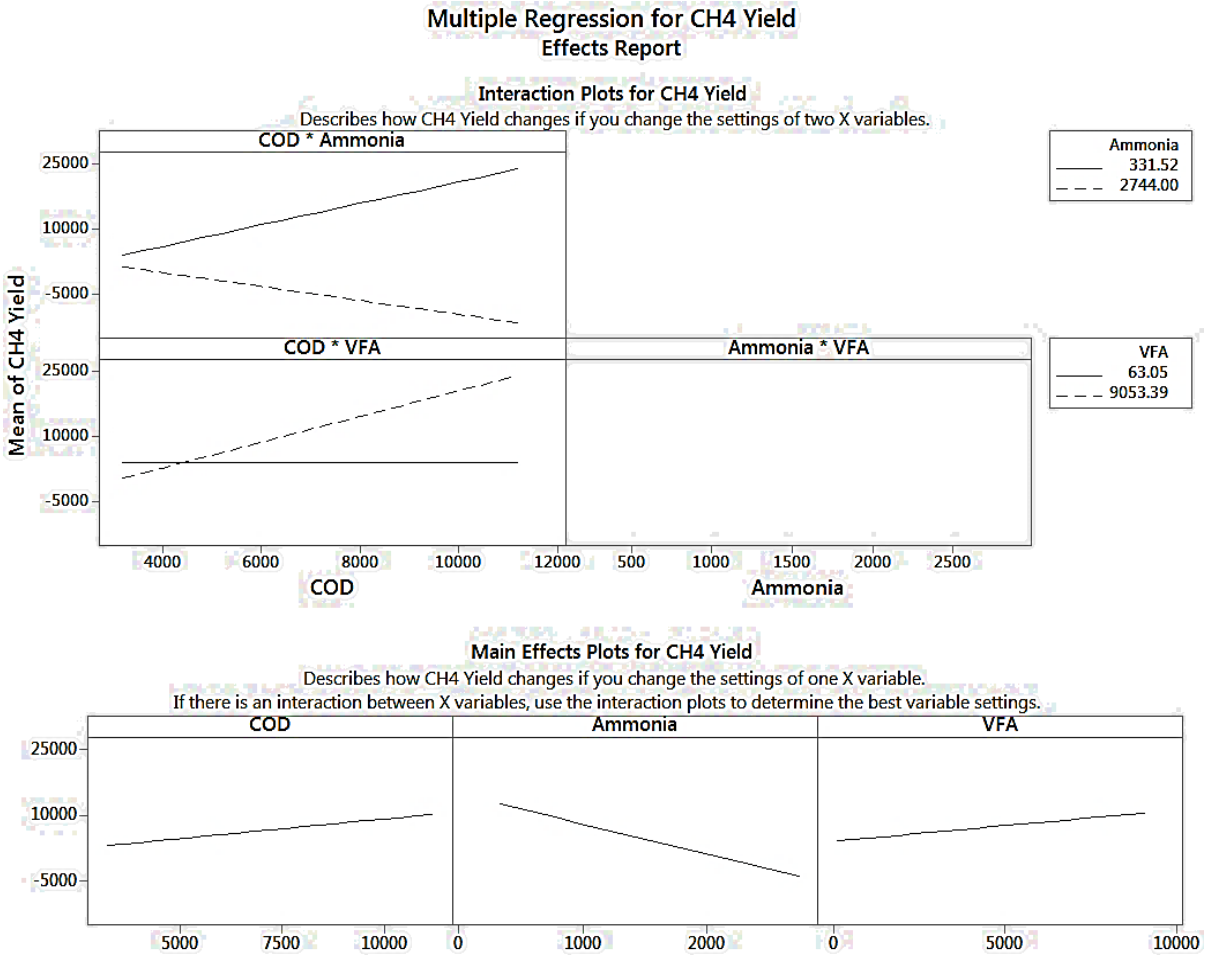

Figure 6: Main effects plot for mesophilic reactor (MR 1) on methane production

It can be seen that for predictors X_1 and X_2 they have a positive gradient, and as their value increases, the methane production increases up to a maximum concentration of 9.1 g L^{-1} for VFAs, with the COD concentration (11.2 g L^{-1}) having the most effect. The effect of ammonia shows a negative correlation, with low concentration having a higher impact on methane production. The interaction terms showed quite an interesting phenomenon, for optimal process conditions, ammonia concentration up to 331 mg L^{-1} will give an increasing

methane production with increasing COD concentration up to 4.0 – 10.0 g L^{-1}, whereas with high ammonia concentration approximately ~ 2.7 g L^{-1}, the reverse is the case, producing low methane as the COD concentration increases. The relationship between COD and VFAs shows that optimal VFAs concentration up to 9.1 g L^{-1} will aid methane yield with increasing COD concentration. At a low VFA concentration of 63.1 g L^{-1}, the methane yield seems to be almost constant producing virtually very low methane as the COD concentration increases. This is not unexpected as VFAs are intermediates produced in AD process which serves as precursors for methane formation [18], but higher concentration can cause inhibitory and detrimental effects which could lead to a slow production of biogas [19].

4.2. Surface and contour plots analysis for mesophilic reactor

The results of the interaction effects on surface and contour plots for the mesophilic reactor MR 1 are shown in Figure 7. From the COD/ammonia interaction surface plot, better methane production will be obtained with a COD > 5.0 g L^{-1}, and an ammonia concentration up to 1.0 g L^{-1}. Increase in COD concentration up to 10.0 g L^{-1} with a lower concentration of ammonia will eventually produce a low methane yield. Higher concentration of ammonia close to 2.0 g L^{-1} with increase in COD concentration will cause a sharp drop in the methane production with a negative response, indicating inhibition of the process. The interaction between COD and VFAs has a very low impact on the process and indicates that as VFAs concentration increases up to 10.0 g L^{-1}, COD < 5.0 g L^{-1} will tend to give process optimal conditions, yielding high methane production. The impacts the interactions of ammonia and VFAs has on the predicted outcome of methane production shows that as the ammonia concentration reduces to below 1.0 g L^{-1} with a corresponding increasing VFAs up to 10.0 g L^{-1} more methane production will be achieved. The results of the contour plots gave a more refined and clearer picture of the interactions of the process parameters, which is similar to the observations from the surface plot. Results of the curvature of the interactions between COD and ammonia shows that lower ammonia concentration below 500 mg L^{-1} with an increasing COD concentration up to 10.0 g L^{-1} will give a high yield up to 25 – 50 L CH_4 / reactor but within a very low margin. COD range 5.0 ≤ 10.0 g L^{-1}, and ammonia 1.5 ≤ 2.5 g L^{-1} regions will give a good range of optimal methane production. Process inhibition is likely to occur when the COD > 5.0 g L^{-1} and ammonia concentration > 2.5 g L^{-1} producing a negative response in the process. The curvature for the impact of COD and VFA interactions shows optimal conditions will be achieved at COD values 5.0 ≤ 9.0 g L^{-1}, and VFAs of 6.0 ≤ 8.0 g L^{-1} without any process instability during the continuous digestion of the macroalgae feedstock. The interaction effect between ammonia, and VFAs from the curvature results shows high methane production at VFAs up to 8.0 g L^{-1} when the concentration of ammonia is < 1.0 g L^{-1}. At ammonia concentration > 2.0 g L^{-1} even with VFAs concentration in the range of 2.0 – 8.0 g L^{-1} process inhibition is likely to occur with a negative response in methane production.

4.3. Model equation generation: Thermophilic temperature

For the thermophilic reactor TR 1, the result of the quadratic second–order multiple regression in form ANOVA is also shown in Table 3.

Table 3: Analysis of Variance (ANOVA) for Response surface model at thermophilic temperature (TR 1)

Source	DF	Adj SS	Adj MS	F-Value	P-Value	Rank
Model	9	494217822	54913091	60.47	0.000	Significant
COD	1	23442024	23442024	25.81	0.004	Significant 1
VFA	1	57117	57117	0.06	0.812	8
Ammonia	1	9391511	9391511	10.34	0.024	4
COD*COD	1	9925304	9925304	10.93	0.021	3
VFA*VFA	1	420622	420622	0.46	0.526	6
Ammonia*Ammonia	1	2453196	2453196	2.70	0.161	5
COD*VFA	1	334892	334892	0.37	0.570	7
COD*Ammonia	1	1676834	16716834	18.41	0.008	2
VFA*Ammonia	1	3696	3696	0.00	0.952	9
Error	5	4540550	908110			
Total	14	498758372				

$R^2 = 0.9909$; Adj.$R^2 = 0.9745$

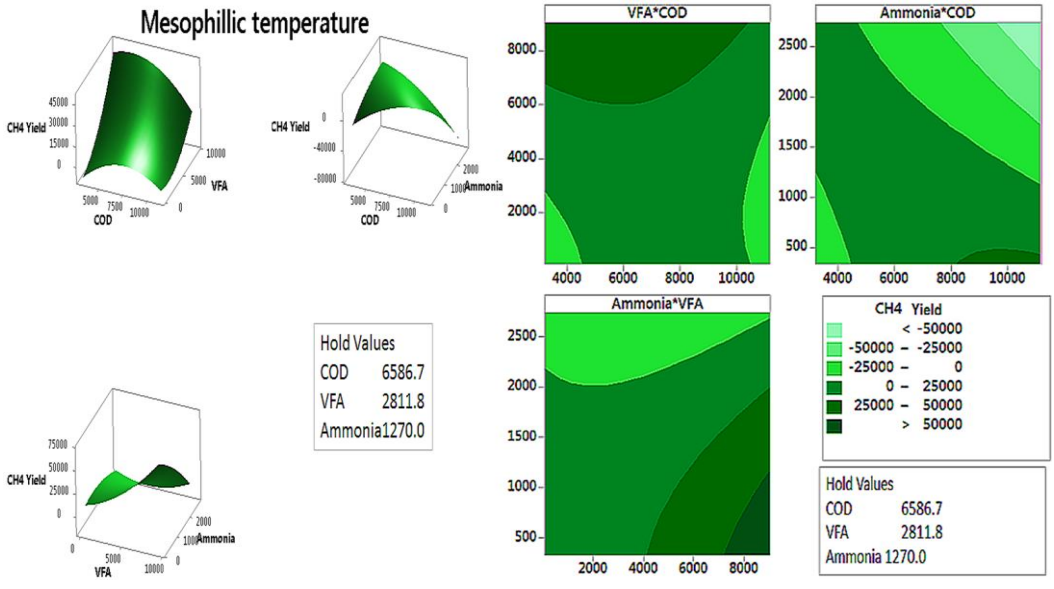

Figure 7: Surface and contour plots for mesophilic reactor (MR 1).

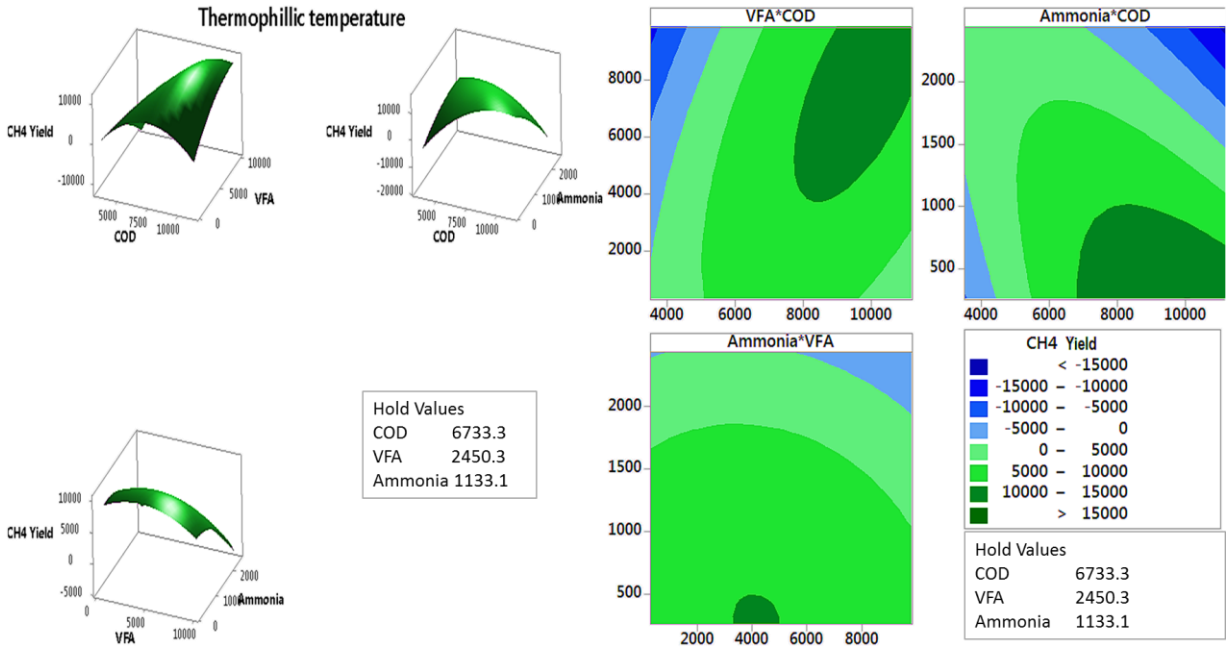

Figure 8: Surface and contour plots for thermophilic reactor (TR 1)

The following quadratic equation and its 2-way interactions terms were generated, Equation 2.

CH_4 production (Y) Equation 2

$$= -33561 + 9.33\,X_1 - 1.07\,X_2 + 16.34\,X_3 - 0.000471\,X_1^2 - 0.000110\,X_2^2 - 0.00209\,X_3^2 + 0.000300\,X_1 \times X_2 - 0.002185\,X_1 \times X_3 - 0.00009\,X_2 \times X_3$$

Where X_1: COD, X_2: VFA, X_3: Ammonia.

The relationship between Y and the X variables in the model is statistically significant with a $p < 0.000$. The regression coefficient R^2, is 0.99, indicating a perfect fit for the model. Solution to optimal conditions from the model building sequence of the interactive terms using multiple regression gave; X_1: COD = 6.7 g L^{-1}, X_2: VFAs = 2.5 g L^{-1}, X_3: Ammonia = 1.1 g L^{-1}. From the coefficients in Equation 2, and the ranking in Table 3, the predicted impacts of the variables on methane yield is: $X_1 > (X_1*X_3) > X_1\text{^}2 > X_3 > X_3\text{^}2 > X_2\text{^}2 > (X_1*X_2) > X_2 > (X_2*X_3)$, with X_1 (COD) concentration having the most impacts. The impact between COD (X_1^2) and ammonia (X_2) interactions are of the same magnitude.

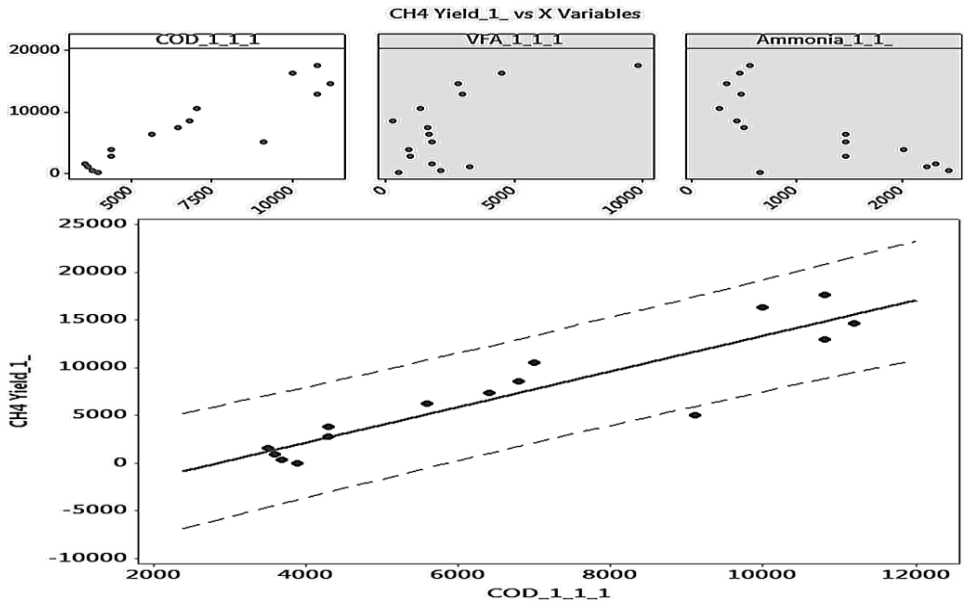

Figure 9: Main effects plot for thermophilic reactor (TR I) on methane yield

Using multiple regression analysis, the main effects and interactions among the various variable in the thermophilic reactor (TR 1) are shown in Figure 9. The predicted impacts of the variables on methane production are strongly related to the COD concentration but not strongly with the VFAs or ammonia concentrations. As the COD increases, the predicted methane production increases. The regression coefficient (0.834) suggests it was adequate to simulate the experimental data, hence, while the COD concentration play a critical in methane production, the other interactions among these parameters did not significantly affect the methane produced in the thermophilic reactor. The results of the surface and contour plots for the thermophilic reactor TR 1 is also shown in Figure 8. The graphs shows from the COD/ammonia interaction, the characteristics of the surface plot is similar to what was obtained in the mesophilic reactor MR 1 (Figure 7). Higher methane production can be obtained with a COD > 5.0 g L^{-1} and an ammonia concentration up to 1.0 g L^{-1}. However, as the COD concentration continues to increase up to 10.0 g L^{-1} with a lower concentration of ammonia, the process will tend to produce less quantity of methane. At high concentration of ammonia close to 2.0 g L^{-1} a sharp drop in methane production will be obtained with a negative response, indicating inhibition of the process. This effect is more pronounce with the mesophilic reactor. At COD < 5.0 g L^{-1}, with a reduction in ammonia concentration <1.0 g L^{-1}, a drop in methane production will also gradually occur. The interaction between COD and VFAs indicates that as VFAs concentration increases up to 10.0 g L^{-1} an increase in COD up to 7.0 g L^{-1} will tend to give process optimal conditions, yielding high methane production. Below, this COD concentration < 5.0 g L^{-1} or above 7.0 g L^{-1}, reduction in VFAs concentrations will tend to lower the methane production, and eventually lead to reactor failure, due to negative output in the biomethane yield. The impacts of the interaction of ammonia and VFAs on the predicted outcome of methane production, shows as the ammonia concentration reduces to below 1.0 g L^{-1} with a corresponding increase in VFAs up to 10.0 g L^{-1}, more methane production will be achieved, but is quickly inhibited, when the ammonia concentration increases up to 2.0 g L^{-1}, tending towards very low methane production. From results of the contour plots, the curvature of the interactions between COD and ammonia shows lower ammonia concentration below 500 mg L^{-1}, with an

increasing COD ≥ 8.0 g L^{-1} will give a high yield up to 15 L CH_4 / reactor. When the COD concentration is > 7.0 g L^{-1}, and ammonia > 1.5 g L^{-1} the process will tend to produce low quantity of methane, leading to an inhibited state where the gas production will be completely seized with a negative output. The curvature for the impact of COD and VFA interactions shows optimal conditions will be achieved at COD values of between 5.0 – 9.0 g L^{-1}, and VFAs concentrations of 6.0 – 8.0 g L^{-1} without any instability to the continuous digestion process. Below, COD < 4.0 g L^{-1} and VFAs > 2.0 g L^{-1}, process inhibition might start to set in, leading to low methane production and outright process failure. The interaction effect between ammonia and VFAs from the curvature results shows optimal gas production at VFAs up to 4.0 g L^{-1}, when the concentration of ammonia is < 500 mg L^{-1}. At ammonia concentration > 2.0 g L^{-1} with VFAs concentration in the range of 6.0 – 8.0 g L^{-1}, process inhibition is likely to occur with a negative response in methane output.

5. Conclusion

Optimisation techniques are normally used in anaerobic digestion process to propose areas where improvements could be made when commercialisation is considered. Optimisation refers to process performance improvement for maximum benefit, and traditionally applied by monitoring the influence of one factor at a time on an experimental response. Experimental results; pH, COD, VFA, Ammonia, and alkalinity were subjected to correlation analysis using matrix plot, and identified correlated parameters were back eliminated, reducing the parameters to COD, VFA, and ammonia which were adequate to simulate the regression model in both the mesophilic and thermophilic reactors. Surface and contour plots were used to describe the optimisation process and to evaluate the effects and interaction of COD, VFA and ammonia on methane production. The model regression analysis generated a second-order quadratic equation in form of ANOVA in both the mesophilic and thermophilic reactors. Solution to optimal conditions from the equation for optimised methane production were derived as X_1: COD = 6.6 g L^{-1}, X_2: VFAs = 2.8 g L^{-1}, X_3: Ammonia = 1.3 g L^{-1} for the mesophilic reactor and X_1: COD = 6.7 g L^{-1}, X_2: VFAs = 2.5 g L^{-1}, X_3: Ammonia = 1.1 g L^{-1} for the thermophilic reactor.

6. Recommendations

Multivariate techniques tools could be used to optimize methane production from anaerobic digestion processes, understanding impacts of process parameters, while predicting optimal process conditions for large scale processes. This could eventually reduce process inhibition and failure with net gain in production cost.

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