Palm oil is one of the main agricultural products in Malaysia as it contributes 49.5% of the total world production [1]. On an average standard palm oil mills, for each tonne of fresh fruit bunch (FFB)
processed, generates about 1 tonne of liquid waste with a pollution load of biochemical oxygen demand of (BOD) 37.5 kg, a chemical oxygen demand the (COD) 75 kg, suspended solids (SS) 27 kg and oil and grease 8 kg [2]. There are currently about 265 active palm oil mills in Malaysia with a combined annual CPO production capacity of about 13 million tonnes [3]. This amounts to a population equivalent of around 80 million in terms of COD. Thus, there is an urgent need to find an efficient and practical approach to preserve the environment while maintaining the economy.

Considering the highly organic character of palm oil mill effluents (POME), the anaerobic process is the most suitable approach for treatment [4]. The common practice of treating POME is by using ponding and/or open digestion tank systems which have particular disadvantages such as: long hydraulic retention times of 45-60 days [5], bad odour, difficulty in maintaining the liquor distribution to ensure smooth performance over huge areas and difficulty in collecting biogas which could have detrimental effects on the environment [6-7].

High-rate anaerobic reactors, that can retain biomass, have a high treatment capacity and hence low site area requirement [8]. POME COD removal efficiencies in excess of 85% have been reported for high rate reactors such as the anaerobic baffled reactor (ABR) [9], the single up-flow anaerobic sludge blanket (UASB) reactor [10], two stage UASB system [11] and membrane anaerobic system (MAS) [12]. In summary, the high rate anaerobic reactors mentioned above are successfully able to treat POME at short HRT.

The UASB reactor exhibits positive features, such as high organic loadings, short hydraulic retention time (HRT) and a low energy demand, especially for POME treatment [10,13]. Suspended and colloidal components of POME in the form of fat, protein, and cellulose have an adverse impact on UASB reactor performance and can cause deterioration of microbial activities and wash out of the active biomass [10,14]. The use of internal packing as an alternative for retaining biomass in the UASB reactor is a suitable solution for the mentioned problems [2-15]. Process instability was observed when a UASB reactor (at HRT of 4 d) and an anaerobic hybrid reactor (AHR) (at HRT of 3.5 d) were operated with high influent COD concentrations of 42500 and 65000 mg/l, respectively [10-15]. Consequently, complete digestion of raw POME without pretreatment demands high HRT, which is not easily achieved due to the high volume of POME produced by the mills. Various pretreatment approaches have been examined for the separation of suspended solids, oil and grease from POME. These include: Chemical coagulation and flocculation [16-18], air flotation simple and skimming [19-20], ultra-filtration [21-22], evaporation [23], centrifugation [20].

The present research is a comparative study of anaerobic digestion of POME which has been physically (primary sedimentation unit) and chemically (chemical coagulation and flocculation pretreated). Results obtained from the high rate digestion of pretreated POME were compared using the response surface methodology (RSM) with respect to the simultaneous effects of two independent operating variables, feed flow rate (QF) and up-flow velocity (Vup).

2. MATERIAL AND METHODS

2.1. Wastewater Preparation Raw POME was collected from a local palm oil mill in Nibong Tebal, Penang, Malaysia. In the first stage, raw POME was pre-settled using an ordinary sedimentation tank. In the second part of this study, raw POME was chemically pretreated to remove suspended solids and residual oil (using a cationic and anionic polymers). The samples were then stored in a cold room at 4 °C. PMOE stored under such conditions has no observable effects on its composition. The characteristics of the raw and pretreated POME are summarized in Table 1.

2.2. Bioreactor and Start Up A laboratory-scale, up-flow anaerobic sludge fixed film (UASFF) reactor was used in this study. The glass bioreactor column was fabricated with an internal diameter of 6.5 cm and a liquid height of 112 cm. Total volume of the reactor was 4980 ml, and the working volume was 3650 ml. The column consisted of three sections; bottom, middle and top. The bottom part of the column, with a height
TABLE 1. Characteristics of the Raw, Pre-Settled and Pretreated POMEa.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Raw POME</th>
<th>Pre-settled POME</th>
<th>Chemically Pretreated POME</th>
</tr>
</thead>
<tbody>
<tr>
<td>BOD₅ (mg/l)</td>
<td>22700</td>
<td>20100</td>
<td>9750</td>
</tr>
<tr>
<td>COD (mg/l)</td>
<td>44300</td>
<td>28640</td>
<td>13880</td>
</tr>
<tr>
<td>Soluble COD (mg/l)</td>
<td>17140</td>
<td>17140</td>
<td>13880</td>
</tr>
<tr>
<td>TVFA (mg acetic acid/l)</td>
<td>2510</td>
<td>2510</td>
<td>2760</td>
</tr>
<tr>
<td>SS (mg/l)</td>
<td>19780</td>
<td>5760</td>
<td>&lt; 20</td>
</tr>
<tr>
<td>Oil and grease (mg/l)</td>
<td>4850</td>
<td>1 630</td>
<td>Negligible</td>
</tr>
<tr>
<td>Total N (mg/l)</td>
<td>780</td>
<td>660</td>
<td>480</td>
</tr>
<tr>
<td>pH</td>
<td>4.05</td>
<td>4.05</td>
<td>4.2</td>
</tr>
</tbody>
</table>

aValues are average of three measurements. The differences between the measurements for each were less than 1%.

of 80 cm was operated as a UASB reactor, the middle part of the column with a height of 25 cm was operated as a fixed film reactor and the top part of the bioreactor served as a gas-solid separator. The middle section of the column was packed with 90 Pall rings with a diameter and height equal to 16 mm. The voidage of the packed-bed reactor was 91.25 % and the specific surface area of the packing material was 341 m²/m³. An inverted funnel shaped gas separator was used to conduct the biogas to a gas collection tank. The UASFF reactor was operated under mesophilic conditions (38 ± 1°C) and the temperature was maintained by circulating hot water through the bioreactor jacket. In order to distribute the feed uniformly in the reactor, an influent liquid distributor was mounted at the base of the column. The inoculum for seeding was an equal proportion mixture of sludge taken from a drainage channel bed of Perai Industrial Zone (Butterworth, Malaysia), digested sludge from a food cannery factory and animal manure. Details regarding the start up procedure can be found elsewhere [2].

2.3. Bioreactor Operation and Experimental Design  The UASFF bioreactor was separately operated with pre-settled and chemically pretreated POME and experiments were designed by Design Expert software (Stat-Ease Inc., version 6.0.6) with two variables, feed flow rate (Qf) and up-flow velocity (V_up) were found to be the most critically independent operating variables which affected the performance of the reactor. The region of exploration for POME treatment was decided as the area enclosed by Qf (1.01, 3.31 l/d) and V_up (0.2, 3 m/h) boundaries for pre-settled POME and Qf (1.01, 7.63 l/d) and V_up (0.2, 3 m/h) for chemically pretreated POME. This would cover an OLR range of 7.9 to 26.0 and 3.8 to 29.0 g COD/l.d for pre-settled and chemically pretreated POME respectively. A steady state was assumed after five turnovers.

In order to carry out a comprehensive analysis of the anaerobic process, 4 dependent parameters were either directly measured or calculated as response. These parameters were total COD (TCOD) removal, effluent total volatile fatty acid (TVFA), effluent bicarbonate alkalinity (BA) and methane yield (YM).

Data analysis was carried out using the response surface methodology (RSM) under general factorial design. The results were completely analyzed using analysis of variance (ANOVA) which was automatically performed by Design Expert Software (ver. 6.0.6). Three dimensional (3D) plots and their respective contour plots were obtained based on the effect of the levels of the two factors. From these three-dimensional plots, the simultaneous interaction of the two factors on the responses was studied. The RSM used in the present study was a Central Composite Face-
2.4. Analytical Methods The following parameters were analyzed according to Standard Methods [24]: pH, alkalinity, TSS, VSS, BOD and COD. Total Kjeldahl nitrogen (TKN) was determined by a colorimetric method using a DR 2000 spectrophotometer (Hach Co. Loveland, Co). Gas chromatographs equipped with a thermal conductivity detector (TCD) and a flame ionization detector (FID) were used for the determination of biogas and volatile fatty acid compositions, respectively [2].

3. RESULTS AND DISCUSSION

Raw POME contains a high concentration of suspended solids (Table 1) which requires long
retention time for satisfactory digestion. A fraction of TSS which is not digestible is gradually accumulated in the reactor by attaching to it the sludge granules in the UASFF reactor it causes reduction in process efficiency. From a practical point of view and according to various studies [2,10,11,15], the oil-bearing suspended solids need to be removed (partially or completely) before anaerobic treatment in order to have a reliable, stable and efficiently high rate anaerobic process.

3.1. POME Digestion

3.1.1. TCOD Removal The effect of the variables on TCOD removal efficiencies are shown in Figure 1a and b as contour plots for pre-settled and chemically pretreated POME, respectively. Since total suspended solids (TSS) of the pre-settled POME contained 5760 mg/l, a fraction of the OLR is in suspended solids whereas in the chemically pretreated POME, the entire OLR is solubel. In an overall comparison, the trend of changes in TCOD removal efficiency was quite similar for both conditions. The TCOD removal (%) decreased with an increase in QF while the rate of TCOD removal (g COD/l.d) was increased (Table 2), due to an increase in the diffusion rate of substrate at higher substrate concentration [26-27].

A stable TCOD removal efficiency of 83.5 % was achieved at the highest QF (3.31 l/d, corresponding to OLR of 26 g COD/l.d) for pre-settled POME whereas only 62.2 % TCOD removal was achieved with chemically pretreated POME at QF of 7.63 l/d (corresponding to OLR of 29 g COD/l.d) and that also was coupled with process instability. It was found that at the same OLR (center points, Vup from 0.2 to 3 m/h), despite 33% of OLR in the pre-settled POME being suspended solids, which needs to be hydrolyzed first and greater COD removal efficiency (90-94 %) was achieved compared to chemically pretreated POME the COD removal efficiency was in the range of (82-88 %). This may be attributed to possible inhibitory effects of the polymers which were applied for chemical pretreatment.

3.1.2. Effluent TVFA The VFA concentration is a key indicator of system performance. Figure 2a and b depict the effects of the variables on the effluent VFA for the pre-settled and the chemically pretreated POME, respectively. The highest concentrations of VFA, as intermediates, was
found during overloading conditions when they were 553 mg/l for the pre-settled POME at OLR of 26 g COD/l.d and 1613 mg/l for the chemically pretreated POME at OLR of 29 g COD/l.d. In both experiments, the role of $V_{up}$ in the system recovery at a high $Q_F$ was very significant due to its effects on recycled alkalinity and contact between substrate and biomass [28]. From the 3D graph, at comparable OLRS i.e. 16.95 g COD/l.d ($Q_F = 2.16$ l/d) for pre-settled POME and 16.42 g COD/l.d ($Q_F = 4.32$ l/d) for chemically pretreated POME, the VFA concentration for the two cases were also similar. It showed that there was a balance between acetogenesis and methanogenesis in the systems at this OLR. In the chemically pretreated POME digestion, process upset occurred as methanogenic bacteria could not metabolize the VFA as fast as they were produced; resulting in a possible reduction in pH. In the chemically pretreated POME digestion, process upset occurred when methanogenic bacteria could not metabolize the VFA as fast as they were produced; resulting in a possible reduction in pH. This condition ($Q_F = 7.63$ l/d, $V_{up} = 0.2$ m/h) had a strong influence on the biogas quality, increasing the CO$_2$ percentage (75.22 %). Similar behavior was observed in a secondary UASB reactor treating piggery waste at an HRT of 1 day and an influent COD of 10189 mg/l [29].

3.1.3. Effluent BA Figure 3a and b show the interactive effects of $Q_F$ and $V_{up}$ on bicarbonate alkalinity (BA). It was understood that the BA was produced through POME digestion reactions as no chemical was added to create alkalinity. Effluent BA values for pre-settled POME were greater than the values for chemically pretreated POME within the tested range of the two variables.

From Figure 3a, the maximum level for BA was predicted to be the region where $Q_F$ and $V_F$ were relatively high (the values larger than center point) while it was obtained from the middle part of the design space in Figure 4b. At the highest OLR and the lowest $V_{up}$ (corresponding to $Q_F$ of 3.31 l/d in Figure 3a and 7.63 l/d in Figure 4b both at $V_{up}$ 0.2 m/h), BA was obtained as 1720 and 960 mg CaCO$_3$/l for the pre-settled and the chemically pretreated POME, respectively. In this condition ($V_{up} = 0.2$ m/h), the BA concentration was not high enough to avoid process upset for chemically
pretreated POME while the process remained stable for pre-settled POME. The effect of $V_{\text{up}}$ on system recovery was more significant at a high feed flow rate due to more alkalinity recycling.

### 3.1.4. Methane Yield

Figure 4a and b represent the simultaneous effects of the variables on the methane yield as contour plots. It shows that a simultaneous decrease in the variables yielded an increase in the response for both pre-settled and chemically pretreated POME. It was found that the yield values for the pre-settled POME (Figure 4a) were greater than the values for the chemically pretreated one. The highest level of the yield was 0.34 and 0.33 for pre-settled and chemically pretreated POME, in that some order where $Q_f$ and $V_{\text{up}}$ were 1.01 "l/d" and 0.2 m/h respectively. It was also found that a minimum retention time longer than 1.5 and 2.2 d, respectively, for the pre-settled and chemically pretreated POME digestion was needed to achieve high methane yield.

### 4. CONCLUSION

Response surface methodology was a potent tool to compare the results obtained from the anaerobic treatment of the two different types of pretreated POME with different characteristics. At an OLR of about 16.5 g COD/l.d (center point), despite 33% of OLR in the pre-settled POME being suspended solids, greater COD removal efficiency was achieved compared to chemically pretreated POME. Effluent BA values for pre-settled POME were greater than the values for chemically pretreated POME within the tested range of the two variables. The highest level of the yield was 0.34 and 0.33 for pre-settled and chemically pretreated POME, in that some order where $Q_f$ and $V_{\text{up}}$ were 1.01"l/d" and 0.2 m/h respectively.

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Figure 4. DESIGN-EXPERT plot. Contour plot of methane yield representing the effect of the feed flow rate and up-flow velocity (a) Pre-settled POME (b) Chemically pretreated POME.

6. REFERENCES