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# Effect of inoculum to substrate ratio on the performance of modified anaerobic inclining-baffled reactor treating recycled paper mill effluent

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## ABSTRACT

Three start-up techniques were investigated to evaluate the suitability of the bench-scale modified anaerobic inclining-baffled reactor (MAI-BR) for the treatment of recycled paper mill effluent (RPME) and to achieve an improved understanding of the inoculum to substrate ratio (ISR). The ISR ratios used were 3.53, 1.17, and 10.63 g volatile suspended solids (VSS)/g chemical oxygen demand (COD) for the first, second, and third start-ups, respectively. On a 30-d duration, the first start-up succeeded in removing 72% of the COD with an effluent pH of 6.2 and a methane production of 0.076 L/d. The second start-up was considered unfavorable after 16 d because of low methane production and effluent pH level. The third start-up with a 21-d duration was the best because of its 87% COD removal, 6.82 effluent pH, and 0.164 L/d methane production. The effluent volatile fatty acid (VFA)/alkalinity ratios were concurrently varied as 0.56, 0.45, and 0.034 for the first, second, and third start-ups, respectively. Subsequently, the VSS/TSS ratio of 0.78–0.86 formed in the reactor was sufficient to control biomass washout. The third start-up demonstrated that an ISR of more than 10 g VSS/g COD is an important factor that leads to a successful and efficient start-up operation. The batch feeding rate must be as low as 0.1 g COD/L d. Furthermore, the VFA/alkalinity ratio of the third start-up was excellent. The bench-scale unique design of the MAI-BR showed good performance in the RPME treatment within a period of less than one month.

*Keywords:* Anaerobic digestion; Recycled paper mill effluent (RPME); Bench-scale; Modified anaerobic inclining-baffled reactor (MAI-BR); Start-up techniques

## 1. Background

Anaerobic reactors are a popular technology for the treatment of industrial wastewater, and they are broadly used around the world. The main advantages of anaerobic reactors are their low energy requirement and minimal production of excess sludge. They can also be combined with the use of other processes to effectively remove organic matter and nutrients. This combination is a promising alternative to the traditional treatment of industrial wastewater [1].

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Anaerobic baffled reactors (ABRs) are efficient anaerobic reactors developed in the 1980s. The simple structure, high organic loading rate (OLR), low operating costs, and excellent performance of each diverse anaerobic microbe achieved by the separation of biophases are some of the advantages of ABRs [2]. Research on the use of ABRs to treat high and low organic wastewater has increased in recent years. ABRs present a number of advantages for the hydrolysis process. Because of the several small compartments in ABRs, phase-split anoxic conditions can occur in each compartment along the wastewater stream. ABRs are also resistant to shock load and toxicity, and their other benefits are the partial separation of acidogenesis and methanogenesis and the absence of short streaming, jams, or back streaming [3].

A slow start-up procedure is a significant difficulty in ABR operation, and it is crucial to the overall wastewater treatment process because of the slow growth rate of methanogen micro-organisms. The start-up of anaerobic reactors is determined by the primary transient period, which is characterized by operational instabilities. The start-up of an anaerobic reactor is a relatively fragile process that depends on various factors, such as reactor configuration, operating conditions, available inoculum, and wastewater composition. The start-up of ABR and the factors that affect it have been investigated in depth by Barber and Stuckey [4].

Meanwhile, the inoculum to substrate ratio (ISR) has been shown to affect the consumption of volatile fatty acids (VFAs), methane production rate, and methane yield. The ISR is generally presented on the basis of volatile suspended solids (VSS). The ISR affects the duration and occurrence of the lag phase (extracellular hydrolysis), VS/chemical oxygen demand (COD) reduction, methanogenesis, and susceptibility of micro-organisms to inhibitory effects [5]. The ISR is a major parameter that affects the process of anaerobic digestion, and it should be higher than 10 in terms of the VSS to COD for a constant process.

Dealing with the long and difficult start-up periods of anaerobic reactors can often be frustrating for researchers in the laboratory. The ISR is a crucial parameter, but it is unfortunately excluded by many researchers from the experimental design [6]. Meanwhile, few studies have reported on the start-up variation techniques of ABRs [7–9], which include treatment of the recycled pulp and paper mill wastewater with the use of anaerobic technology [10,11].

Few relevant data on the parameters of the effluents released from recycled paper mills are currently available. The most important task is the assessment of the effluents of recycled paper mills to design specific treatment systems for recycling mills. In this regard, this study aims to explore different start-up strategies to determine the importance of the ISR on the performance of a bench-scale modified anaerobic inclining-baffled reactor (MAI-BR) in the wastewater treatment of recycled paper mill effluent (RPME).

## 2. Materials and methods

### 2.1. The bench-scale modified anaerobic inclining-baffled reactor

The laboratory-scale MAI-BR used in this study was fabricated with the use of plastic polypropylene. The MAI-BR details have been reported elsewhere [12], and the reactor schematic diagram is shown in Fig. 1. With a total effective volume of 35 L (calculated without baffles and packing materials), the MAI-BR was constructed with dimensions of 80 cm in length, 15 cm in width, and 30 cm in height (without water jacket). The MAI-BR consisted of five chambers, each separated by a modified vertical baffle, with the bottom portion of the hanging baffles bent to direct the flow into upflow chambers.

Each modified baffle has its own characteristics (form/shape) to facilitate good contact and mixing of the feed RPME and sludge at the bottom part of the MAI-BR. About 50% of the total volume of the second and third compartments was filled with 7 L of packing materials. The reactor had an attached water jacket to maintain the reactor temperature at 37°C. Centrifugal pumps were used to control the influent feed rate to the first chamber of the reactor system.

### 2.2. Substrate and seeding inoculum

For the seeding process, flocculant anaerobic palm oil mill effluent (POME) sludge was obtained from Malpom Palm Industries Bhd, Penang, Malaysia. The POME sludge was kept in closed containers to avoid biological contamination by air. The microbial activities of the seed sludge were then tested in three sets of serum bottle by analysis of biogas production after 7 d. The gas produced contained 11.3% methane, which indicates that the sludge is active and favorable to use as a source of anaerobic micro-organism during the MAI-BR start-up operation for the RPME treatment.

A new sludge sample was collected, immediately used, and fed to the reactor. The POME seed sludge had solid contents of 4,135 mg total suspended solid (TSS)/L, 3,535 mg VSS/L, and 32,137 mg COD/L. The substrate used in this study was wastewater obtained

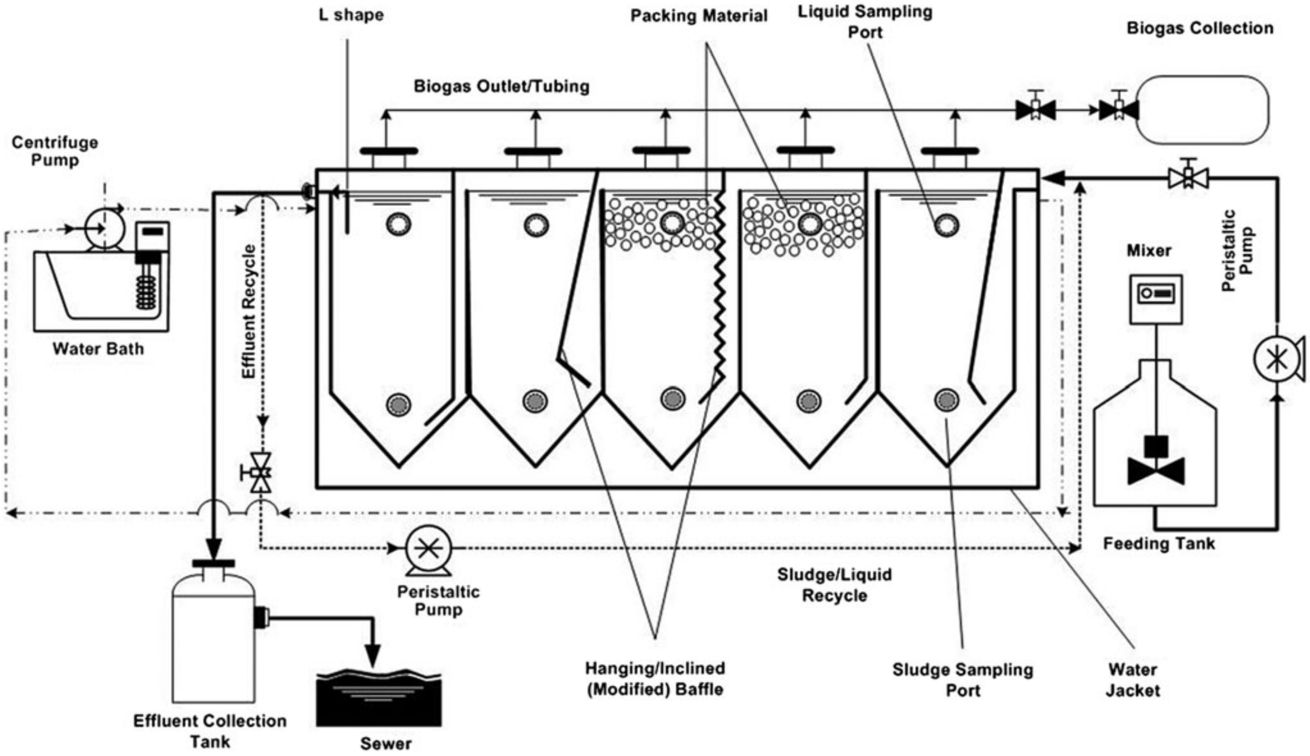

Fig. 1. The modified anaerobic inclining-baffled reactor (MAI-BR) schematic diagram.

from Muda Recycled Paper Mill, Penang, Malaysia. The substrate was kept in a cooling room at 4˚C until use in the seeding process. The RPME was diluted four times before being directly fed to the reactor.

### 2.3. Start-up strategy

The following three consecutive stages were followed in each start-up:

- Acclimation of the inoculum POME seeding sludge to the reactor condition: The reactor was seeded with POME sludge only without adding any source of nutrients (wastewater).
- Acclimation of the inoculum micro-organism that was adapted in the reactor to the RPME substrate: Daily batch feeding using a low OLR of the RPME substrate was conducted.
- Acclimation of the reactor micro-organism that was formed from the two previous stages to the reactor operation technique (i.e. hydraulic retention time and OLR): Continuous feeding of the RPME substrate was performed. Table 1 shows additional details about each start-up operation procedure and reactor conditions.

During the first start-up, the reactor was seeded with only 3.5 L (10% of the total effective reactor volume) of the POME anaerobic sludge, which gave an ISR of 3.53 (g VSS/g COD). Then, the reactor was operated on a daily batch stage by manual addition of 3.5 L (10% of the total effective reactor volume) of the RPME to the reactor as a substrate. The raw RPME COD concentration, which was diluted to 1,000 mg COD/L, gave an OLR of 0.1 g COD/L d. During the batch feeding, the RPME was fed to the reactor through the sampling ports at the top of each chamber and the inoculum–substrate mixture did not flow from one chamber to the next, the MAI-BR served as five independent batch reactors. After the MAI-BR reached its capacity, the continuous stage was started with an influent COD concentration of 1,000 mg/L, which gave an OLR of 0.2 g COD/L d. In this stage, effluent recycling was not applied during the continuous operation.

To investigate the effect of batch feeding on the MAI-BR, the second start-up was conducted by addition of 10% of the seeding sludge to the reactor; this gave an ISR of 1.17 (g VSS/g COD). The reactor was then operated on a high daily batch stage by addition of 10.5 L (30% of the total effective reactor volume) of

Table 1
Reactor operation procedure during each start-up technique

| | Stage | Period (d) | OLR (g COD/L d) | HRT (d) | Inoculum vol. (L) | ISR (g VSS/g COD) |
|---|---|---|---|---|---|---|
| First start-up | Stage 1 | 1 | – | – | 3.5 | 3.53 |
| | Stage 2 (Batch mode) | 1–9 | 0.1 (Intermittent) | 10 | – | |
| | Stage 3 (Continuous mode) | 10–30 | 0.2 | 5 | – | |
| Second start-up | Stage 1 | 1 | – | – | 3.5 | 1.17 |
| | Stage 2 (Batch mode) | 1–3 | 0.3 (Intermittent) | 3 | – | |
| | Stage 3 (Continuous mode) | 4–16 | 0.2 | 5 | – | |
| Third start-up | Stage 1 | 1 | – | – | 10.5 | 10.63 |
| | Stage 2 (Batch mode) | 1–7 | 0.1 (Intermittent) | 10 | – | |
| | Stage 3 (Continuous mode) | 8–21 | 0.2 | 5 | – | |

the RPME to the reactor as a substrate. The RPME concentration was controlled to 1,000 mg COD/L, which gave an OLR of 0.3 g COD/L d. After the MAI-BR reached its capacity, the continuous phase was started with an influent COD concentration of 1,000 mg/L, which gave an OLR of 0.2 g COD/L d.

Because the start-up of an anaerobic reactor depends on various factors (e.g. available inoculum), the third start-up was performed by addition of 10.5 L of the seeding sludge (i.e. 30% of the total effective reactor volume), which gave an ISR of 10.63 (g VSS/g COD). The daily batch stage with 1,000 mg COD/L concurrently followed, which gave an OLR of 0.1 g COD/L d of the RPME. After the MAI-BR reached its capacity, the continuous stage was started with an influent COD concentration of 1,000 mg/L, which gave an OLR of 0.2 g COD/L d. During the reactor start-up, the samples (including biogas) from the effluent were collected every 2 d throughout the operational period until a steady state condition was achieved.

### 2.4. Sampling and analytical methods

Biogas production, biogas composition, and pH were the parameters monitored during the batch and continuous feeding, whereas COD removal efficiency was monitored during the continuous feeding technique. To evaluate the performance of the MAI-BR during the steady state of each start-up, eight parameters, which included COD, biological oxygen demand (BOD), pH, alkalinity, fatty acids, TSS, VSS, and floc size, were tested. These various parameters were evaluated for each compartment, the influent, and the effluent.

For sample analysis, triplicate samples were collected for each reading and analyzed twice to enhance the precision of the results. Only the average value was reported throughout this study. The repeatability of the experimental data was found to be sufficiently high, with a relative error less than 5% between repeated analyses. These analyses included BOD, pH, alkalinity, total solids (TS), suspended solids (SS), and VSS, which all accorded with standard methods [13].

The COD was measured with DR-2800 Spectrophotometer according to the reactor digestion method adopted from Jirka and Carter [14]. The total VFA was determined also with DR-2800 Spectrophotometer according to the esterification method by Montgomery et al. [15]. The microbial floc size was measured with Malvern Particle Size Analyzer Model 2000. Heavy metals were analyzed with an inductively coupled plasma-optical emission spectrometry model (Varian 715-ES). Methane ($CH_4$) was determined with Shimadzu GC-FID with a ProPack N column.

## 3. Results and discussion

### 3.1. The RPME as a substrate and seed inoculum

Table 2 shows the physicochemical characteristics of the RPME and POME. The RPME results showed a relatively high COD value of 3,812 mg/L and a $BOD_5$ value of 1,789 mg/L, which reflect a $BOD_5$/COD ratio of 0.49. A $BOD_5$/COD relationship of 0.49 indicates that the wastewater is of high-strength organic type and has a potential to increase in strength with time. In addition, the wastewater is of high-strength biodegradable type, so it is suitable for anaerobic treatment [16,17]. Because of its relatively neutral pH

Table 2
Physical–chemical characterization of substrate (RPME) and seed inoculum (POME)

| Parameter | RPME | POME |
|---|---|---|
| pH | 6.6 | 8.3 |
| Floc size | 326 | – |
| Temperature | 40 | 32 |
| Chemical oxygen demand (COD) | 3,812 | 32,137 |
| Biochemical oxygen demand (BOD) | 1,789 | 14,578 |
| $BOD_5/COD$ | 0.49 | 0.45 |
| Alkalinity | 340 | 1,755 |
| Volatile fatty acids (VFAs) | 566 | 7,324 |
| Ammonia ($NH_4$) | 0.3 | 228 |
| Total solids (TS) | 4,814 | 13,540 |
| Total dissolved solids (TDS) | 2,465 | 9,405 |
| Total suspended solids (TSS) | 2,349 | 4,135 |
| Total volatile solid (VSS) | 1,967 | 3,535 |

Note: Parameters are in mg/L except pH, $BOD_5/COD$, Temperature in ℃, and floc size in μm.

(6.6) and an alkalinity of 340 mg/L, the wastewater was used without any alkaline adjustment. It also contained a relatively high VFA of 566 mg/L, TS of 4,814 mg/L, total dissolved solids of 2,465 mg/L, TSS of 2,349 mg/L, and total volatile solids of 1,967 mg/L.

The metalloids' elements of the RPME and POME were also recorded; these data were reported previously [3]. Most of metals' concentrations exceeded Malaysian standards according to the Environmental Quality Act of 1974 [18]. The metals with the highest concentrations that are present in the effluent stream were Ca (399 mg/L), Mg (12 mg/L), and Fe (2.39 mg/L). The high metalloids concentration shows that these effluents are not suitable for discharge to the water stream because they will increase the inorganic load in water bodies [19].

### *3.2. Start-up performance of the MAI-BR during batch and continuous feeding*

The first start-up was conducted with 10% of the seeding sludge of the total effective volume, and the daily batch phase started from day 1 until the MAI-BR reached its capacity on day 9. The continuous phase started on day 10 until a steady state was reached on day 30. The second start-up was conducted with 10% of the seeding sludge of the total volume and daily batch feeding of 30% for 3 d. The continuous feeding started on day 4. After a few days, MAI-BR performance significantly deteriorated, and the reactor was soured on day 16. The poor quantity of the starting inoculum (a low ISR of 1.17) and the high initial batch loading rate (0.3 g COD g/d) were assumed to be the reasons for the performance deterioration. Therefore, the inoculum of the third start-up was increased to 30% of the seeding sludge of the total effective reactor volume. The batch feeding proceeded for 7 d, whereas the continuous feeding technique continued until a steady state was reached within 21 d.

#### *3.2.1. COD removal efficiency*

At this start-up stage, the influent COD concentration of 3,812 mg/L was used after dilution to 1,000 mg/L without further increase to actual amount. In addition, the calculated COD removal efficiency was based on the diluted amount of 1,000 mg/L. Batch feeding OLRs were fixed to 0.1 g COD/L d for the first and third start-ups and were changed to 0.3 g COD/L d for the second start-up. During the continuous feeding period, the OLR was fixed to 0.2 g COD/L d for all the start-ups. Fig. 2 shows the variation in COD removal efficiency during the continuous feeding at different start-up techniques. COD removal was found to be a function of operating conditions and ISR.

The COD removal rate increased by the time a steady state was reached on days 30, 16, and 21 for the first, second, and third start-ups, respectively. All start-up techniques achieved high COD removal efficiencies of 72, 70, and 87% for the first, second, and third start-ups, respectively. Comparison of the COD removal at each start-up showed that the COD reduction efficiency was directly proportional to the ISR with the obtainment of a high COD removal efficiency of 87% at the third start-up. The effluent COD concentrations were higher (282 mg/L) at the first start-up than at the third one (139 mg/L).

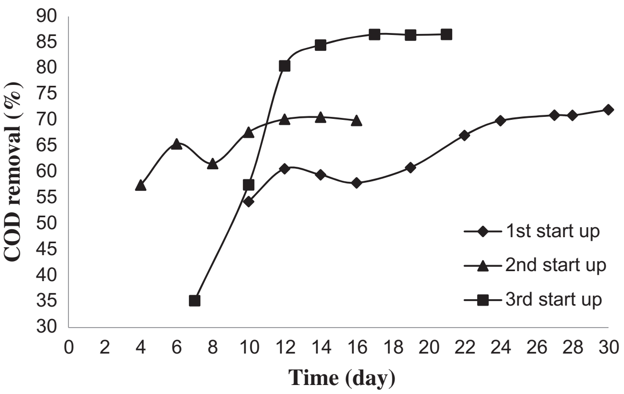

Fig. 2. COD removal efficiency profile during continues operation time.

Turkdogan et al. [20] reported the start-up of an upflow anaerobic sludge blanket (UASB) reactor that treats pulp and paper. The reactor was seeded with anaerobic granules, and the start-up period was 29 d. The overall COD removal efficiency of the UASB was reported to be 60%. In another study on the effect of the ISR, Eskicioglu and Ghorbani [21] indicated that in an ISR range of 3.67–0.46 g/g on the basis of VS, the kinetic constants (*k*) for COD and VS removals significantly decreased, as a result demonstrating initial substrate inhibition or reactor overloading. At the highest organic loading (ISR of 0.46 g/g), degradation was completed in 22 d, and digesters achieved 76–86% of COD removal.

### 3.2.2. Methane composition

Fig. 3 illustrates the methane production in the reactor system during the batch and continuous treatment of the RPME. Low but varying amounts of methane production were observed during each start-up because of the different ISRs. At the first start-up, the methane composition was found to increase daily during the batch phase, but it decreased during the continuous phase because of the different feeding techniques in the reactor. However, methane composition increased with a slight fluctuation. In the 30-d duration of the first start-up, the methane composition increased from 0.002 to 0.076 L $CH_4$/d. At the same time, methane production was observed to reach its steady state on day 22.

For 16 d of the second start-up, the methane content reached only 0.036 L $CH_4$/d. Because 30% of the seeding sludge of the total effective reactor volume was used during the third start-up, the batch feeding continued for 7 d, and a similar pattern was observed as that of the first start-up, albeit with a high methane composition. The continuous feeding technique continued until a steady state was reached in 21 d. In addition, the third start-up demonstrated that the ISR was necessary to achieve a high methane composition (i.e. 0.164 L $CH_4$/d) within a short time (21 d). The methane yields also varied as 0.009, 0.004, and 0.015 for the first, second, and third start-ups, respectively.

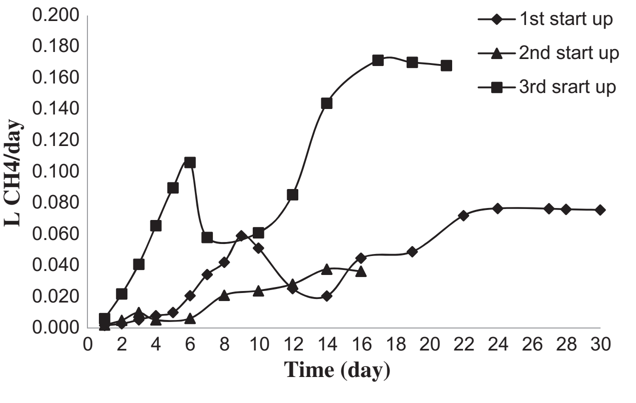

Fig. 3. Methane composition profile as L $CH_4$/d during batch and continues operation time.

A similar result proved that ISR can affect the methane yield or methane production rate. The kinetics of methane production were dependent on the concentration of the inoculums used; however, the final yield was the same [22]. A relatively high COD removal rate was achieved, whereas a small amount of methane gas was produced. Therefore, a partial amount of the COD was believed to be utilized for the new cell production of micro-organisms, rather than for biogas production at the start-up period. As regards the matter, Krishna et al. [23] conducted COD mass balance in ABR. Only 31.6–39.7% of the total COD was found to be utilized for methane gas production; the rest was used for biomass production, uncounted COD, and other losses as dissolved $CH_4$ in the effluent.

### 3.2.3. pH

One of the important observations in the anaerobic process is with regard to the pH level. Fig. 4(a) shows the comparison of effluent pH profiles during three operation techniques. No chemicals were added during the start-up to maintain the pH levels of the MAI-BR. The pH level of the first start-up fluctuated (from 7.28 to 6.12), which indicated the adjustment of the micro-organisms to maintain the desired pH levels (6.6 to 7.7) for an anaerobic reactor [24].

Throughout the second start-up, the performance of the pH level deteriorated and decreased from 7.77 to 5.95 until a further decrease was already unfavorable because of the unrecovered pH level during a period of 16 d. An accumulation of VFAs consequently occurred. Another study has shown that a low ratio of inoculums to the feed can lead to the inhibition of methanogenesis and results in the accumulation of VFAs [5]. Meanwhile, an excellent performance was demonstrated by the third start-up. The pH level decreased from 7.62 to 6.84 and remained around this level until the steady state condition was achieved on day 21.

To evaluate performance in terms of pH characteristic level throughout the reactor, the pH profiles of the influent, compartments 1–5, and the effluent of the first, second, and third start-ups shown in Fig. 4(b)–(d), respectively, were analyzed. All three start-ups showed

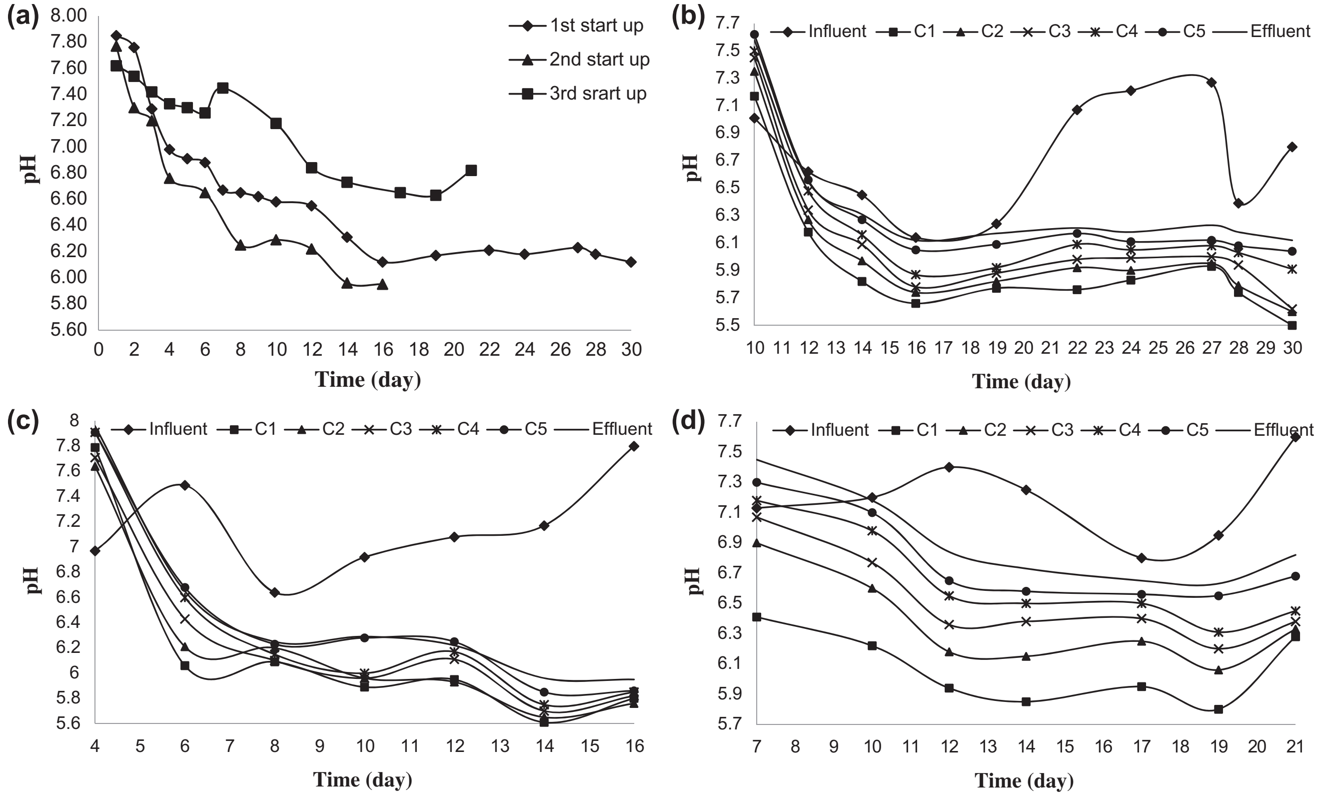

Fig. 4. pH level profiles (a) effluent pH during batch and continues operation time of each start-up, (b) compartmental pH level of the first start-up, (c) compartmental pH level of the second start-up and (d) compartmental pH level of the third start-up.

the same pattern: the pH level linearly increased toward the end of the reactor with the different pH levels.

Although the pH levels slightly decreased during the operational period, high COD removal efficiencies confirmed the ability of the MAI-BR configuration to overcome the adverse effect of pH. One possible explanation is the relatively low HRT (5 d) applied to the reactor system during the continuous feeding phase. However, the third start-up showed a stable performance compared with the others. Eskicioglu and Ghorbani [21] reported the same result in which a reactor system with a low ISR of 0.46 (high loading rate) had VFA accumulations and a slight decrease in pH, whereas other systems with a high ISR (low loading rate) had negligible VFAs during the first 8 d of biodegradation.

### 3.3. Start-up performance of the MAI-BR during steady state

The steady state was initially identified during a less than 10% change in the removal efficiency of organic matter. When the steady state was achieved, the sludge and supernatant liquor samples were separately collected from each compartment, influent, and effluent to evaluate the performance of the MAI-BR. Eight parameters, which included COD, BOD, pH, alkalinity, fatty acids, TSS, VSS, and floc size, were tested. The results for all the three start-ups are discussed in the following sections.

#### 3.3.1. BOD and COD

Table 3 presents the variations in COD and BOD concentrations for each start-up. COD and BOD showed the same concentration pattern that varied according to each start-up. In compartment 1, the COD removal efficiencies were 61, 60, and 76%, whereas the BOD removal efficiencies were 46, 40, and 81% for the first, second, and third start-ups, respectively. The maximum COD removal efficiencies were 71, 70, and 90%, whereas the BOD removal efficiencies were 71, 69, and 91% for the first, second, and third start-ups, respectively. These removal efficiencies

Table 3
The steady state compartmental performance of MAI-BR at each start-up

| | COD (mg/L) | | | BOD (mg/L) | | | VSS (mg/L) | | | Fatty acid (mg/L) | | | Alkalinity | | | pH | | | Floc size (μm) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1st | 2nd | 3rd | 1st | 2nd | 3rd | 1st | 2nd | 3rd | 1st | 2nd | 3rd | 1st | 2nd | 3rd | 1st | 2nd | 3rd | 1st | 2nd | 3rd |
| Influent | 988 | 991 | 1,017 | 516 | 514 | 502 | 255 | 244 | 392 | 92 | 104 | 107 | 200 | 198 | 193 | 6.8 | 7.8 | 7.6 | 170 | 196 | 180 |
| C1 | 328 | 332 | 243 | 280 | 308 | 63 | 600 | 274 | 108 | 181 | 115 | 67 | 155 | 165 | 160 | 5.8 | 5.5 | 6.3 | 164 | 159 | 160 |
| C2 | 322 | 330 | 150 | 277 | 272 | 22 | 275 | 208 | 242 | 213 | 100 | 20 | 170 | 170 | 183 | 5.8 | 5.6 | 6.3 | 160 | 129 | 29 |
| C3 | 316 | 322 | 111 | 232 | 237 | 34 | 225 | 114 | 196 | 163 | 101 | 7 | 175 | 160 | 198 | 5.8 | 5.6 | 6.4 | 150 | 110 | 80 |
| C4 | 298 | 315 | 107 | 238 | 216 | 32 | 195 | 108 | 230 | 152 | 80 | 4 | 210 | 160 | 223 | 5.9 | 5.9 | 6.5 | 148 | 107 | 37 |
| C5 | 294 | 304 | 103 | 193 | 184 | 38 | 145 | 98 | 194 | 133 | 73 | 6 | 225 | 175 | 230 | 6.4 | 5.9 | 6.7 | 130 | 99 | 9 |
| Effluent | 282 | 296 | 99 | 150 | 162 | 44 | 139 | 84 | 54 | 121 | 77 | 8 | 215 | 170 | 235 | 6.4 | 6 | 6.8 | 112 | 82 | 4 |

generally increased toward the rear of the reactor, and a large part of the COD and BOD was removed in the two front compartments.

These findings on compartmental COD removal are comparable with those of Torabian et al. [25]. They found that the maximum COD removal occurred in the first compartment (up to 51.2%), and the rest of the COD was removed in the other compartments (up to 27.4%). Because the COD decreased in the subsequent compartment, the substrate utilization rate of the micro-organisms in the preceding compartments was reduced, and this led to a low removal efficiency. This phenomenon is attributed to bacterial kinetics that decrease substrate concentrations and thus cause low growth rates [26].

Subsequently, this observation indicates that a small compartment number can be suitable to treat low-strength wastewater in the MAI-BR. However, the compartments in the rear of the MAI-BR played an important role in the fermentation of the RPME. Although different conditions were applied, the design of the MAI-BR, especially the modified baffles, improved COD and BOD removal. The data on COD and BOD removal efficiencies show that the third start-up technique achieved the highest removal rate. The reactor performed well because of the sufficient ISR (10.63). Similarly, Barber and Stuckey [8] reported that the loading rate in the initial phase of the start-up of an anaerobic reactor must be as low as 10 g VSS/g COD.

### 3.3.2. pH level

Table 3 shows that the pH values of the first, second, and third start-ups were found to be in the range of 5.76–6.44, 5.5–5.95, and 6.28–6.82, respectively. During the first start-up, the pH in compartments 1–4 was in the acidic range, whereas a neutral pH range was observed in the other compartments. During the second start-up, the overall pH levels in the reactor were in the acidic range and did not recover. During the third start-up, the pH in the reactor was observed to have slightly increased. Acidogens in front compartments ferment fatty acids, monosaccharides, and amino acids to simple organic acids. Thus, production of simple organic acids and specifically acetic acid resulted in reduction of pH [27].

In addition, the reduction of pH levels in front compartments and its increase at consecutive compartments indicate appropriate separation of acidogens and methanogens in the system. Accordingly, pH is decreased in MAI-BR front compartments as a result of VFA accumulation. Malakahmad et al. [28]

observed an increase in pH in successive compartments due to decreasing VFA concentration with increasing alkalinity wherein methanogens convert the hydrogen and acetic acid to methane gas and carbon dioxide and causes rise in pH.

Similar results were reported by Torabian et al. [25] who found that the pH in the initial compartments was the lowest with the occurrence of acidogenesis and acetogenesis. Despite the variation in the pH of feed wastewater, the effluent pH levels were constant at a particular start-up. The constant effluent pH values implied the effective consumption of VFAs by *methanogens*. Intriguingly, reductions in biogas production and methane composition (% $CH_4$) were observed to be associated with the decrease in pH.

For all the three start-ups, the MAI-BR showed the ability to partially separate the various phases of anaerobic catabolism. Fast-growing bacteria capable of growth at high substrate levels and reduced pH were dominant in the front compartment of the reactor (the acidification zone). By contrast, slow-growing scavenging bacteria that grow well at a high pH were dominant toward the end of the reactor (the methanogenic zone) [4]. Compared with the first and second start-ups, the third start-up obtained a high pH level led to its good performance.

### 3.3.3. Alkalinity and fatty acids

Alkalinity levels indicate potential anaerobic process performance. Low values of effluent alkalinity signal impending reactor failure. A large number of studies on the importance of alkalinity and VFAs in anaerobic digestion have been conducted and are reported in the literature [29]. During the start-up period (i.e. the time of acclimatization at new conditions), the alkalinity levels were observed to be low at the first compartments (Table 3). The low effluent alkalinity coincided with the low COD reduction efficiencies. However, once the start-up period is over, the reactor performance at a particular start-up technique stabilizes. The alkalinities of the first, second, and third start-ups were found to be in the range of 155–215, 155–170, and 160–235 mg/L, respectively. At the third start-up, the effluent alkalinity levels were 16–20% more than the influent alkalinity levels. This increase in alkalinity can be caused by the formation of carbonates and bicarbonates in the reactor [30].

At the same time, phase separation will cause the VFA concentration in each chamber to decrease toward the rear of the reactor. Table 3 depicts that the high level of VFA in compartments 1 and 2 can be caused by the high activity of hydrolytic and acidogenic bacteria compared with methanogenic bacteria. However, the VFA level in the next set of compartments decreased. During the third start-up, the VFA concentration significantly declined compared with that in the other two start-ups, especially in the rear of the reactor; this phenomenon can be caused by methanogenic bacteria becoming active with the increased availability of inoculum [5]. Different microenvironments create a domain of specific bacteria population.

Similar trends were observed by Malakahmad et al. who found that the hydrolysis, acidogenesis, and acetogenesis were occurred in the initial compartments because the accumulation of fatty acids was restricted only at the front compartment of the reactor and did not affect the methanogenesis that occurred in the rest of the compartments [28]. Malakahmad and Yee [27] also observed an increase in alkalinity in consecutive compartments due to the decrease inVFA concentration wherein methanogens convert the hydrogen and acetic acid to methane gas and carbon dioxide and causes rise in pH.

Therefore, VFA and alkalinity are reversely related. The ratio of VFA to the total alkalinity is a measure of the stability of an anaerobic process because this ratio indicates a balance between acidogenesis and methanogenosis within the reactor. For proper anaerobic functioning, the balance should be maintained well below 0.5 [31]. The effluent ratios were observed to vary among 0.56, 0.45, and 0.034 at the first, second, and third start-ups, respectively. The ratio of the third start-up was excellent.

### 3.3.4. TS and VSS

Table 3 shows the variation in TSS and VSS in each compartment with the start-up variation. The TSS concentration of the diluted influent ranged from 309 to 500 mg/L with 77–80% in VSS form. Up to 45% TSS removal was observed at the first start-up, and it increased to 87% at the third start-up. The TSS concentrations in the treated effluent were 174, 98, and 67 for the first, second, and third start-ups, respectively. The TSS concentrations in the treated effluent were observed to be unaffected by the varying TSS concentrations in the influent wastewater at a particular start-up. At the third start-up, the VSS effluent concentration was less than those at the first and second start-ups. The VSS reduction efficiency was 46, 66, and 86% at the first, second, and third start-ups, respectively. At the third start-up, the biomass washout was unnoticed because of the poor structure of the MAI-BR, the provision of an adequate number of chambers, and the development of highly settleable sludge.

Chelliapan et al. [10] indicated that low levels of VSS were washed out from a stage anaerobic reactor because the reactor baffles prevented solid washout. The control of biomass washout is affected by granule size distribution (GSD) in the sludge. Vlyssides et al. [32] attributed GSD to granule density, diameter, and VSS/TSS ratio. A VSS/TSS ratio of 0.78–0.86 observed in the MAI-BR sludge was necessary and sufficient to control biomass washout. Furthermore, separation of individual sets of compartments helped in reducing biomass carryover to the next set of compartments. Granulation was unnecessary for optimal performance in the ABR, but various studies have demonstrated that the granules could appear in the reactor under favorable conditions [33].

Furthermore, a low biomass washout is a result of the use of packing media, which affected the achievement of an improved sludge retention time. In an anaerobic baffled reactor that treats black liquor, Kennedy et al. [34] reported that the attachment of high concentrations of biomass (in terms of VSS) to filter media minimized the risk for biomass washout. In addition, the biomass film and the total quantity in the system are highly affected by process configuration and the characteristics of the support media. On the basis of this result, the MAI-BR configuration promotes the formation of granules and populations of filamentous bacteria developed in the rear compartments of granulation, rather than being the initial nuclei for granule formation.

### *3.3.5. Floc size*

Table 3 shows the compartmental floc sizes for each start-up. At the steady state, the average floc sizes after 30, 16, and 21 d were 150 μm, a decrease of 121 μm, and a decrease of 63 μm for the first, second, and third start-ups, respectively. At the first start-up, the flocs in compartment 1 grew to 164 μm, whereas the flocs in the rest of the compartments showed small average sizes of 147 μm. Compared with those in the second start-up, the flocs in compartment 1 grew to 159 μm, whereas those in the rest of the compartments were reduced to an average size of 111 μm. At the third start-up, the flocs in compartment 1 grew to 160 μm, whereas the flocs in the rest of the compartments linearly decreased to an average size of 39 μm.

During the first start-up, a low COD level, which results in less gas production, can be a possible reason for the decrease in floc size toward the end of the reactor. Although this remains to be proven, a dip toward the rear can be attributed to very low substrate levels, which result in low bacterial growth and small flocs. A consequent reduction in floc size, which was most extensive in compartments 4 and 5, was observed in the second and third start-ups. Floc size has been hypothesized to be mainly dependent on hydraulic shear, channeling, and flow patterns, as well as to the mass transfer driving force. In addition, one would expect an increased microbial growth and a concomitant enlargement in floc size toward a certain maximum limit that is controlled by mass transfer limitations in the middle of the flocs if the feeding rate is slow [35].

In the third start-up, the decrease in particle size, which was most severe in the second compartment because of gas mixing effects, was probably caused by hydraulic shear. This effect was subsequently increased in compartments 3–5 because of the high levels of gas mixing. The small particles were eventually washed out altogether, and a profile developed in which the particle size grew to a maximum that approached the center of the reactor. In this study, the floc size seemed to be a function of gas production, hydraulic shear, COD levels, and biomass washout. Saritpongteeraka and Chaiprapat [26] reported that the hydraulic load may have resulted in the movement of methane-producing biomass, which tends to be light and does not form flocs well, to the next set of compartments or out of the reactor.

## 4. Conclusion

This study showed that during the start-up period, the MAI-BR successfully removed COD, TDS, TSS, TS, and VSS inside the RPME. A 21-d duration of the third start-up was successful in achieving 87% COD removal, an effluent pH of 6.82, and methane production of 0.164 L/d. The optimum ISR of the RPME and POME was 10.63 g VSS/g COD. The increased seeding inoculum volume encouraged methanogenic growth and was manifested by a reduction in VFA effluent concentrations, an increase in COD removals, and an increase in system pH. A high batch-fed rate was observed to be harmful to the MAI-BR process. However, the low continuous OLR of 0.2 g COD/L d is part of the start-up strategy, and the reactor will be further transited to the target loading rate of 0.33–4 g COD/L d. Therefore, the operation of the MAI-BR led to a complete biological degradation of organic matter and a good adaptation of the biomass for substrate degradation.

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## Abbreviations

| | | |
|---|---|---|
| ABR | — | anaerobic baffled reactor |
| MAI-BR | — | modified anaerobic inclining-baffled reactor |
| CABR | — | carrier anaerobic baffled reactor |
| SAR | — | stage anaerobic reactor |
| UASB | — | upflow anaerobic sludge blanket |
| RPME | — | recycled paper mill effluent |
| POME | — | palm oil mill effluent |
| OLR | — | organic loading rate (g/L d) |
| HRT | — | hydraulic retention time (d) |
| VFA | — | volatile fatty acids (mg/L) |
| ISR | — | substrate to inoculum ratio (g VSS/g COD) |

## References

[1] S.Ç. Ayaz, L. Akça, Ö. Aktaş, N. Fındık, İ. Öztürk, Pilot-scale anaerobic treatment of domestic wastewater in upflow anaerobic sludge bed and anaerobic baffled reactors at ambient temperatures, Desalin. Water Treat. 46 (2012) 60–67.

[2] S.R. Hassan, H.M. Zwain, I. Dahlan, Development of anaerobic reactor for industrial wastewater treatment: An overview, present stage and future prospects, J. Adv. Sci. Res. 4 (2013) 07–12.

[3] H.M. Zwain, I. Dahlan, Biological treatment of recycled paper mill wastewater using modified anaerobic inclining-baffled bioreactor (MAIB-R), in: H.A. Aziz, A. Mojiri (Eds.), Wastewater Engineering: Advanced Wastewater Treatment, International Journal of Scientific Research (IJSR) Publications, Penang, 2014, pp. 72–86, doi: 10.12983/1-2014-03-01.

[4] W.P. Barber, D.C. Stuckey, The use of the anaerobic baffled reactor (ABR) for wastewater treatment: A review, Water Res. 33 (1999) 1559–1578.

[5] F. Xu, J. Shi, W. Lv, Z. Yu, Y. Li, Comparison of different liquid anaerobic digestion effluents as inocula and nitrogen sources for solid-state batch anaerobic digestion of corn stover, Waste Manage. 33 (2013) 26–32.

[6] G. Esposito, L. Frunzo, F. Liotta, A. Panico, F. Pirozzi, Bio-methane potential tests to measure the biogas production from the digestion and co-digestion of complex organic substrates, Open Environ. Eng. J. 5 (2012) 1–8.

[7] Z. Guochen, L. Jianzheng, H. Junguo, L. Weiguang, M. Chao, The start-up and operational performance of anaerobic baffled reactor (ABR) treating high-concentration sugar-producing wastewater, in: Proceedings of the 3rd International Conference on Bioinformatics and Biomedical Engineering, ICBBE 2009, IEEE, Beijing, China, 2009, pp. 1–4.

[8] W.P. Barber, D.C. Stuckey, The influence of start-up strategies on the performance of an anaerobic baffled reactor, Environ. Technol. 19 (1998) 489–501.

[9] L.H. Freese, D.C. Stuckey, Influence of seed inoculum on the start-up of an anaerobic baffled reactor, Environ. Technol. 21 (2000) 909–918.

[10] S. Chelliapan, S.B. Mahat, M.F.M. Din, A. Yuzir, N. Othman, Anaerobic digestion of paper mill wastewater, Iranica J. Energy Environ. 3 (2012) 85–90.

[11] E.N. Nyanchaga, M.A. Elkanzi, Characteristic strength and treatability of a recycled paper mill wastewater in a UASB reactor, J. Civ. Eng., JKUAT, 8 (2002) 61–77.

[12] H.M. Zwain, N.Q. Zaman, H.A. Aziz, I. Dahlan, A novel design concept of modified anaerobic inclining-baffled reactor (MAI-BR) for wastewater treatment application, J. Inst. Ind. Appl. Eng. 2 (2014) 85–90.

[13] APHA, AWWA and WEF, Standard methods for the examination of water & wastewater, 22nd ed., American Public Health Association (APHA), American Water Works Association (AWWA) & Water Environment Federation (WEF), Washington, DC, 2012.

[14] A.M. Jirka, M.J. Carter, Micro semiautomated analysis of surface and waste waters for chemical oxygen demand, Anal. Chem. 47 (1975) 1397–1402.

[15] H.A.C. Montgomery, N.S. Thom, J.F. Dymock, The rapid colorimetric determination of organic acids and their salts in sewage-sludge liquor, Analyst 87 (1962) 949–955.

[16] H.M. Zwain, S.R. Hassan, N.Q. Zaman, H.A. Aziz, I. Dahlan, The start-up performance of modified anaerobic baffled reactor (MABR) for the treatment of recycled paper mill wastewater, J. Environ. Chem. Eng. 1 (2013) 61–64.

[17] H.M. Zwain, N.Q. Zaman, H.A. Aziz, I. Dahlan, Evaluation of lignin removal from recycled paper mill effluent (RPME) under different start-up's steady state conditions of modified anaerobic inclining-baffled reactor (MAI-BR), Curr. Environ. Eng. 1 (2014) 60–63.

[18] DOE, Environmental Quality Act 1974 and Regulations, Environmental Quality (Industrial Effluent) Regulations (PU (A) 434), Department of Environment, Malaysia, 2009.

[19] S.R. Hassan, H.M. Zwain, N.Q. Zaman, I. Dahlan, Recycled paper mill effluent treatment in a modified anaerobic baffled reactor: Start-up and steady-state performance, Environ. Technol. 35 (2014) 1–6.

[20] F.I. Turkdogan, J. Park, E. Evans, T. Ellis, Evaluation of pretreatment using UASB and SGBR reactors for pulp and paper plants wastewater treatment, Water Air Soil Pollut. 224 (2013) 1–8.

[21] C. Eskicioglu, M. Ghorbani, Effect of inoculum/substrate ratio on mesophilic anaerobic digestion of bioethanol plant whole stillage in batch mode, Process Biochem. 46 (2011) 1682–1687.

[22] C. González-Fernández, P.A. García-Encina, Impact of substrate to inoculum ratio in anaerobic digestion of swine slurry, Biomass Bioenergy 33 (2009) 1065–1069.

[23] G. Krishna, P. Kumar, P. Kumar, Complex wastewater treatment using an anaerobic baffled reactor, Environ. Prog. 26 (2007) 391–398.

[24] B.E. Rittmann, P.L. McCarty, Environmental Biotechnology: Principles and Applications, McGraw-Hill, New York, NY, 2001.

[25] A. Torabian, S. Abtahi, M. Amin, S. Momeni, Treatment of low-strength industrial wastewater using anaerobic baffled reactor, Iran. J. Environ. Health Sci. Eng. 7 (2010) 229–240.

[26] K. Saritpongteeraka, S. Chaiprapat, Effects of pH adjustment by parawood ash and effluent recycle ratio on the performance of anaerobic baffled reactors treating high sulfate wastewater, Bioresour. Technol. 99 (2008) 8987–8994.

[27] A. Malakahmad, W. Yee, Production of energy from palm oil mill effluent during start-up of carrier anaerobic baffled reactor (CABR) equipped with polymeric media, J. Jpn. Inst. Met. 93 (2014) 505–510.
[28] A. Malakahmad, F. Lahin, W. Yee, Biodegradation of high-strength palm oil mill effluent (POME) through anaerobes partitioning in an integrated baffled reactor inoculated with anaerobic pond sludge, Water Air Soil Pollut. 225 (2014) 1–9.
[29] A. Babaee, J. Shayegan, A. Roshani, Anaerobic slurry co-digestion of poultry manure and straw: Effect of organic loading and temperature, J. Environ. Health Sci. Eng. 11 (2013) 15.
[30] S.Y. Bodkhe, A modified anaerobic baffled reactor for municipal wastewater treatment, J. Environ. Manage. 90 (2009) 2488–2493.
[31] M.I. Baloch, J.C. Akunna, Granular bed baffled reactor (Grabbr): Solution to a two-phase anaerobic digestion system, J. Environ. Eng. 129 (2003) 1015–1021.
[32] A. Vlyssides, E.M. Barampouti, S. Mai, Determination of granule size distribution in a UASB reactor, J. Environ. Manage. 86 (2008) 660–664.
[33] J. Marin, K.J. Kennedy, C. Eskicioglu, Characterization of an anaerobic baffled reactor treating dilute aircraft de-icing fluid and long term effects of operation on granular biomass, Bioresour. Technol. 101 (2010) 2217–2223.
[34] J.F. Kennedy, P.S. Panesar, R. Grover, S.S. Marwaha, Continuous methanogenesis of black liquor of pulp and paper mills in an anaerobic baffled reactor using an immobilized cell system, J. Chem. Technol. 81 (2006) 1277–1281.
[35] S. Nachaiyasit, D.C. Stuckey, The effect of shock loads on the performance of an anaerobic baffled reactor (ABR): 1 Step changes in feed concentration at constant retention time, Water Res. 31 (1997) 2737–2747.