*Desalination and Water Treatment*
www.deswater.com

doi: 10.1080/19443994.2015.1119758

57 (2016) 21350–21363
September

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# Effect of inoculum source and effluent recycle on the start-up performance of a modified anaerobic inclining-baffled reactor treating recycled paper mill effluent

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Received 27 July 2015; Accepted 25 October 2015

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## ABSTRACT

In this study, three start-up techniques of modified anaerobic inclining-baffled reactor (MAI-BR) were performance to understand the effect of inoculum source and effluent recycle on the treatment of recycled paper mill effluent (RPME). Flocculant anaerobic sludge from a palm oil mill pond and digested anaerobic sludge from a sewage treatment plant were inoculated in the first and second start-up/phase1, respectively. Results show that high chemical oxygen demand (COD) removals of 93 and 88% were achieved in the first and second start-up/phase1, respectively. The amount of methane produced in the second start-up/phase1 within 15 d was doubled as compared to the amount produced in the first start-up, with a higher methane content of 79% (as compared to 62% in the first start-up). This result indicated that the inoculum source has a significant effect on the reactor performance. The results in the second start-up/phase2 also showed that COD removal and methane production increased up to 94% and 0.52 L $CH_4$/d, respectively. This finding can conclude that the use of effluent recycle has a favorable effect on the performance of MAI-BR. During the first start-up, second start-up/phase1, and second start-up/phase2, the volatile fatty acid/alkalinity compartmental ratios were varied at 0.01–0.06, 0.03–0.43, and 0.02–0.14, respectively, and the overall effluent pH levels were higher than 6.5. The findings indicate the stability of the MAI-BR system in treating RPME.

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

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## 1. Introduction

Anaerobic digestion is an attractive option for waste treatment application, where both pollution control and energy recovery can be achieved. Anaerobic digestion includes the breakdown of biomass by a concerted action of multiple microorganism pathways in the absence of oxygen. Nowadays, numerous biological treatment methods are available and show promising results over the treatment of recalcitrant organic compounds [1]. Compared with the aerobic process, the anaerobic process is considered a more convenient treatment option because of its small quantities of sludge production and low energy requirements. Therefore, anaerobic digestion is increasingly in demand for treating complex industrial wastewater, which can contain toxic materials, and even low concentrations of domestic wastewater [2].

Anaerobic baffled reactor (ABR) is one type of high-rate anaerobic reactor proposed in 1985. Since then, it has increasingly attracted many researchers and operators and had been applied as a promising reactor for municipal and industrial wastewater treatment [3]. Primarily, ABR has inherent advantages over single compartment reactor because its circulation pattern approaches a plug flow reactor [4]. Recently, ABR was reported as an efficient system for the treatment of different wastewaters, including municipal, industrial, and complex [5]. ABR has also been successfully used to treat pulp and paper mill wastewater, as reported by Alighardashi et al. [6], Kennedy et al. [7], and Grover et al. [8].

A successful start-up period is necessary to achieve better treatment efficiency. The development of high and stable chemical oxygen demand (COD) removal efficiencies in the shortest possible time is an important indicator for a successful start-up process of an anaerobic reactor. Different trophic groups can be imbalanced during the start-up process, and may result in reactor failure. Thus, the development of an optimum start-up condition is important. High fluctuations of, for example, pH, temperature, and hydraulic retention time (HRT), must be avoided during start-up, while organic loading rates (OLR) must be consistent [9].

Inoculum selection for anaerobic digestion also plays an important role in organic degradation and biogas production [10]. The inoculum source not only influences the volume of biogas production, but also affects the kinetics of the anaerobic digestion process. The inoculum used to start-up the anaerobic reactor is essential and plays a critical role in controlling acidification. If an active inoculum is used, process failure can be avoided during the start-up phase [11]. The selection of inoculum source is based on the activity, so selecting an active inoculum decreases the amount of inoculum required for the operation of a full-scale reactor and its volume [12].

Effluent recycle also has various advantages and disadvantages on the ABR performance. However, high substrate loading in the front part of the reactor with plug flow characteristics can lead to the accumulation of volatile fatty acid (VFA) and a concomitant decrease in pH, which affects its efficiencies in pollutant removal. High-strength wastewater can more likely expose sensitive bacteria in front compartments to toxic levels of inorganic and organic compounds [13]. However, the overall effects of effluent recycle are still unclear. In practice, the ultimate use of recycle depends on the wastewater type. Effluent recycle becomes beneficial if pH problems are severe. The influent with high levels of toxic material or high loading rates is preferred. The application of effluent recycling should be cautious and only when absolutely necessary.

Currently, small relevant data are available on the parameters of the effluents released from recycled paper mills (RPME). Thus, the main objective of this research is to explore the start-up performance of a modified anaerobic inclining-baffled reactor (MAI-BR) to achieve a shorter start-up period. This study also attempts to investigate the effect of seed inoculum source and effluent recycling on the start-up performance of MAI-BR for the treatment of RPME. Furthermore, RPME treatment is rarely studied using ABRs.

## 2. Materials and methods

### 2.1. MAI-BR

The laboratory-scale MAI-BR used in this study was fabricated using plastic polypropylene. The MAI-BR schematic is shown in Fig. 1, and additional details have been reported elsewhere [14]. The MAI-BR was constructed with dimensions of 80 cm in length, 15 cm in width, and 30 cm in height (without a water jacket), with a total effective volume of 35 L (calculated without baffles and packing materials). It consisted of five chambers, and each chamber was separated by a modified vertical baffle. The lower portion of the hanging baffles was bent to route the flow into the up-flow chambers. Each modified baffle had its own characteristics to facilitate better contact and more efficient mixing of feed RPME and sludge at the lower part of the MAI-BR. Approximately 50% of the total volume of the second and third compartments was filled with 7 L polypropylene pall ring materials. The reactor had an attached water jacket to maintain the reactor temperature at 37°C. Peristaltic pumps were used to

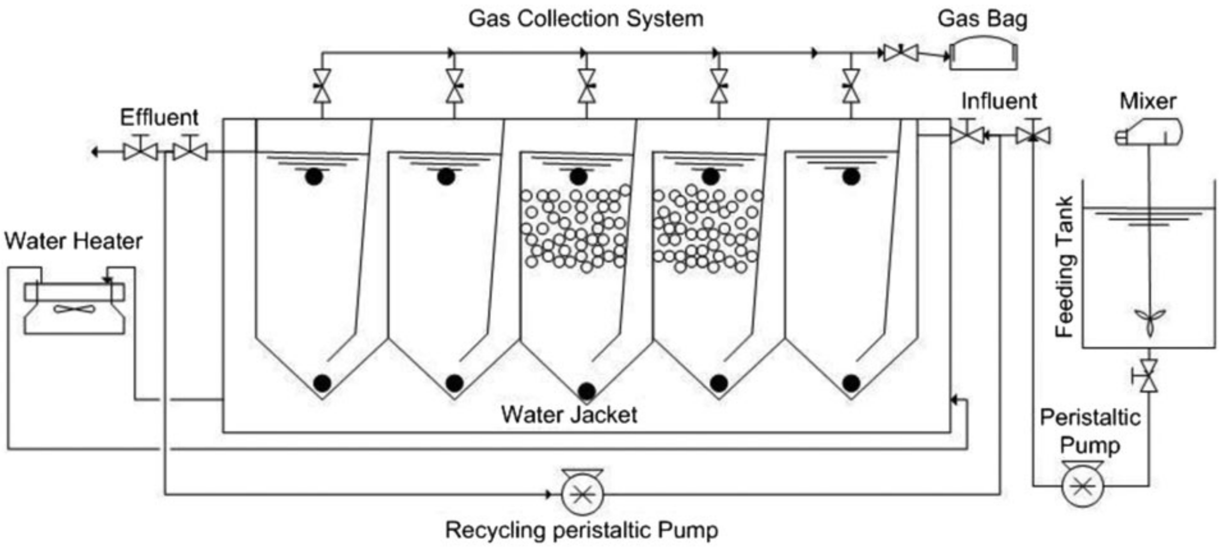

Fig. 1. Schematic diagram of the lab-scale modified anaerobic inclining-baffled reactor (MAI-BR).

control the influent feed rate to the first compartment of the reactor system.

### 2.2. Substrate and seed inoculum

The substrate for this study was wastewater collected from Muda Recycled Paper Mill, Penang, Malaysia and stored in a cooling room at 4˚C. The samples were warmed up to room temperature (27 ± 2˚C) prior to their usage in the seeding process. Thereafter, the RPME was diluted four times before being directly fed to the reactor. Two different seed sludge sources were collected from an anaerobic pond of palm oil mill (POME) of Malpom Sdn Bhd and an anaerobic sludge digester of Jelutong Sewage Treatment Plant (JSTP), Penang, Malaysia for the seed inoculum. These sludge sources were then stored in closed containers to avoid biological contamination by oxygen. The sludge was immediately used and fed to the reactor after collection. The sampling point (location) of POME seed sludge, JSTP seed sludge, and RPME substrate are shown in Fig. 2.

### 2.3. Biomass activity and microbial batch test

Three pairs of 300 mL serum bottles were prepared to test the microbial activities of the different seed sources. The first pair was prepared using 40 mL POME seed and 260 mL RPME substrate, a second pair was prepared using 40 mL JSTP seed and 260 mL RPME substrate, and the third pair contained a 40 mL mixture of the two sludge types (POME + JSTP) and 260 mL RPME substrate. The produced biogas was analyzed every two days for a period of six days.

### 2.4. Start-up strategy

The start-up of an anaerobic reactor depends on different factors, such as operating condition, inoculum-to-substrate ratio (ISR), and inoculum source. The effects of operating condition and ISR were investigated and reported in our previous studies [15–18]. The effects of inoculum source and effluent recycle on the start-up performance were investigated in this study by varying the seed inoculum source and introducing effluent recycle. The first start-up was carried by mixing an equal ratio of POME seed sludge, JSTP, and RPME. In the first part, the MAI-BR was fully seeded, sealed, and stored for five days to enable active acclimatization. In the second part, the MAI-BR was continuously operated with an influent OLR of 0.2 g COD/L d, which corresponded to an influent COD of 1,000 mg/L and HRT of five days. The reactor performance was evaluated in a compartmental-wise fashion after the steady state of the first start-up was attained.

Afterward, the MAI-BR was then shut down and reseeded again using 67% POME sludge and 33% RPME as the second start-up/phase1. Similar to the first start-up procedure, the reactor was continuously operated (until reached steady state condition) with an influent OLR of 0.2 g COD/L d, which corresponded to an influent COD of 1,000 mg/L and HRT of five days. Further compartmental-wise analysis was conducted. The effect of effluent recycle on the MAI-BR start-up was investigated to further emphasize the impact of

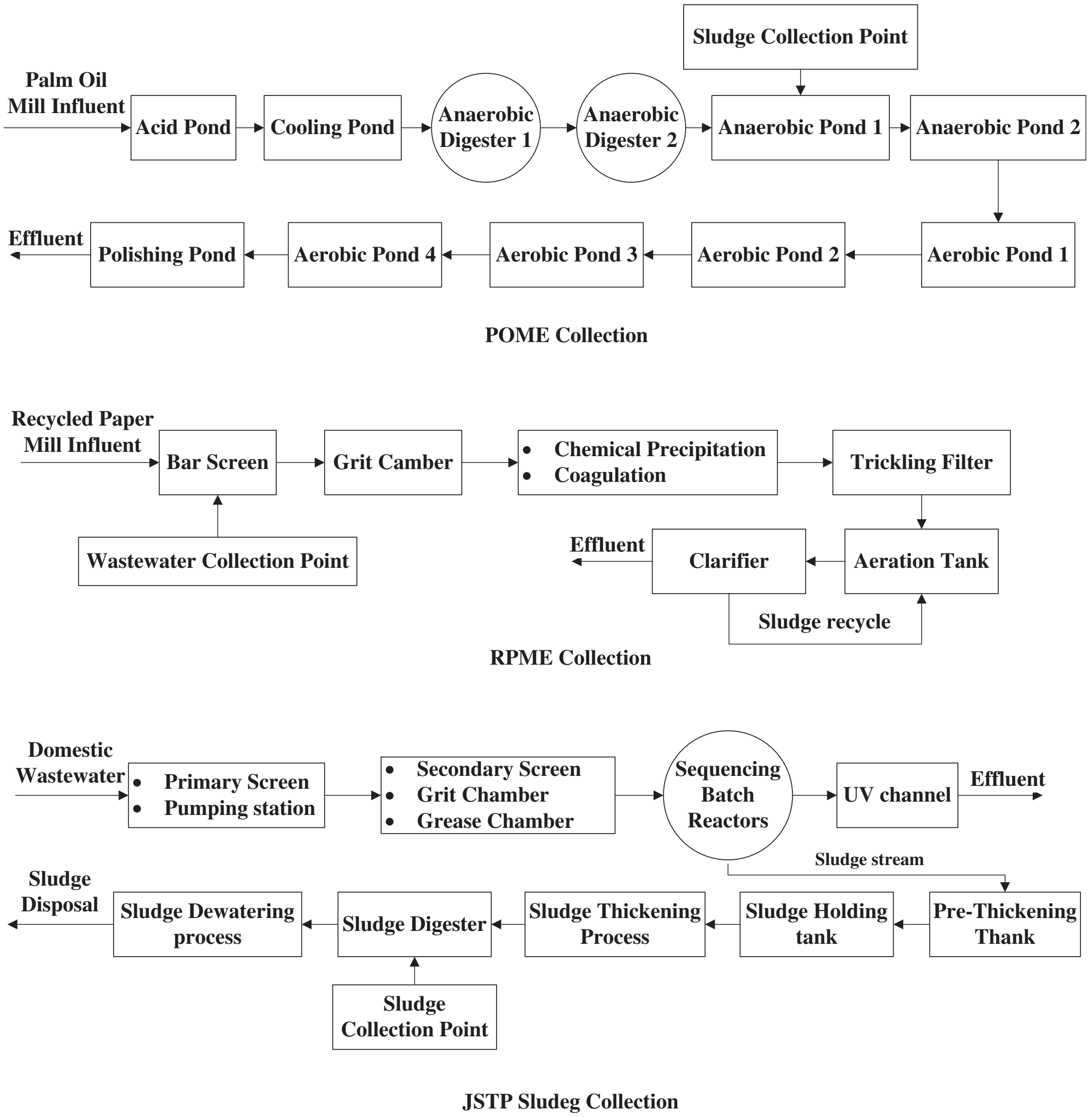

Fig. 2. Layout of treatment process for RPME, POME and sewage, and samples collection points.

reactor operating conditions. The second start-up/phase2 was performed as a continuation of the second start-up/phase1 by applying effluent recycle in a 1:2 ratio. The feeding OLR was increased to 0.33 g COD/L d by applying influent COD of 1,000 mg/L and HRT of three days. Compartmental-wise evaluation was also conducted. Table 1 shows a summary of the start-up operation conditions.

### 2.5. Analytical methods

During the continuous feeding, the biogas, influent, and effluent samples were taken every two days throughout the operational period until the steady-state condition was achieved. The steady state was identified when the change in removal efficiency of organic matter was less than 5% [19]. Compartmental-wise analyses were conducted at the steady-state condition. For the sample analysis, triplicate samples were collected for each reading and analyzed twice to increase 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 between repeated analyses less than 5%. These analyses include biological oxygen demand (BOD), pH, alkalinity, total

Table 1
Reactor operation condition at different start-up technique

| | Time period (d) | OLR (g COD/L d) | HRT (d) | Sludge mixture | ISR | Effluent Recycle ratio |
|---|---|---|---|---|---|---|
| 1st start-up | 1–15 | 0.2 | 5 | 33% JSTP + 33% POME + 34% RPM wastewater | 0.029 | – |
| 2nd start-up/phase1 | 1–15 | 0.2 | 5 | 66% POME + 34% RPM wastewater | 0.066 | – |
| 2nd start-up/phase2 | 15–30 | 0.33 | 3 | Developed mixture | 0.033 | 2 |

suspended solid (TSS), and volatile suspended solids (VSS), all were based on standard methods [20]. The COD and total VFA were measured using a DR-2800 spectrophotometer (HATCH model). Microbial floc size was measured using a Malvern Particle Size Analyzer model 2000. Methane ($CH_4$) concentration was determined using a Shimadzu GC-FID with propack N column.

## 3. Results and discussion

### 3.1. Substrate characteristics and seed inoculum activity

The overall characteristics of the RPME are shown in Table 2. The characteristic results confirm that the biodegradability of the contaminants in the wastewater was high. RPME contains organic nutrients presented as a BOD/COD ratio of 0.49 and a VSS of 1,967 mg/L, which are essential for biological growth. Thus, RPME can be treated biologically and can be fed directly to the reactor [15].

The POME sludge had a TSS of 4,135 mg/L and a VSS of 9.1 g COD/g, whereas the JSTP sludge had a TSS of 23,221 mg/L and a VSS of 2.6 g COD/g. In addition, further physicochemical characteristics of the seeding sludge have been previously reported in Zwain et al. [15]. Three pairs of serum bottles were used and biogas production was analyzed every two days to test the microbial activities of the seeding sludge. The microbial activities were measured in terms of methane concentration. Fig. 3 illustrates that the anaerobic POME sludge yielded a higher methane concentration of 13.03% at only four days, whereas the other two anaerobic sludge samples only produced approximately 3.92 and 0.48% for the mixed and JSTP sludge samples, respectively.

High methane concentration in a short period indicates that these microbes have high activity. Thus, POME sludge is an active and favorable source of anaerobic microorganism to use in the MAI-BR start-up. The purpose of using a mixture of the two different sludge types was to obtain different microorganism species. This approach is only an initial microbial activity test that requires a justification of seed sludge suitability during the start-up of a continuous system to approve the highly active anaerobic microorganism obtained in a batch scale.

### 3.2. Start-up performance of MAI-BR during continuous feeding

Two start-up techniques were operated on continuous feeding until the steady state was achieved. Biogas production, methane concentration, pH profile,

Table 2
The average characteristics of recycled paper mill effluent

| Parameter | Value |
|---|---|
| TDS (mg/L) | 2,465 |
| TSS (mg/L) | 2,349 |
| VSS (mg/L) | 1,967 |
| COD (mg/L) | 3,812 |
| $BOD_5$ (mg/L) | 1,875 |
| BOD/COD | 0.49 |
| pH | 6.6 |
| Alkalinity (mg $CaCO_3$/L) | 430 |
| VFA (mg/L) | 566 |
| Total ammonia (mg/L) | 31 |
| Lignin (mg/L) | 37 |

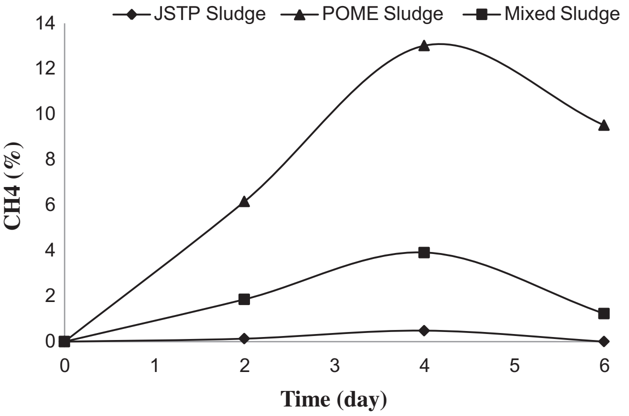

Fig. 3. Methane composition during the microbial activity batch test of different inoculum sources.

and COD removal efficiency were monitored during the continuous feeding technique.

### 3.2.1. COD removal efficiency

Temporal changes in COD removal at each start-up phase of the MAI-BR treating RPME are shown in Fig. 4. The initial influent COD was maintained at 1,000 mg/L, with an OLR of 0.2 g/L d for the first start-up and second start-up/phase1. For the second start-up/phase2, the OLR was increased to 0.33 g/L d. The COD removal in the reactor was comparatively high (more than 90%) within 15 d during the first start-up. The COD removal rate increased from 78 to 92% on day 11 when relatively quick start-up steady state was achieved. Low OLR has better efficiency in COD removal, especially during the start-up of an anaerobic reactor. It was reported that the ABR system cannot sustain an initial high loading rate and its performance sharply deteriorates [7].

In the second start-up/phase1, the COD removal slowly increased from 26 to 33% for the first seven days. The COD removal further increased to 88% for a period of 15 d. The above results on COD removal during start-up are also comparable to other studies on the treatment of high-concentration sugar-producing wastewater using an ABR [21]. They also reported that COD removal rate in the reactor was comparatively low (less than 60%) in the first four days but increased to 85% within 28 d. As a continuation of the second start-up/phase1, the second start-up/phase2 showed that the effluent recycle was compliant to the COD removal and improved up to 94%. The high COD removal efficiency of 94% was observed when the OLR was 0.33 g/L d. The result on the effluent recycle was in line with another study on the treatment of aircraft deicing fluid using ABR [22]. The study found that the recycle improved reactor performance by operating a minimum HRT of 17 h with an acceptable COD removal efficiency of 93%.

The overall results conclude that the first start-up achieved a high COD removal in a short period of time. This condition can be due to the sludge stability that provides more contact between substrates and microorganisms. Nevertheless, the second start-up/phase1 experienced POME sludge floating and sludge overflowing (i.e. the COD removal slightly decreased as compared to the first start-up), but the MAI-BR system had a strong ability to resist sludge washes out. In addition, a high COD removal of 94% in 30 d indicates that no substantial inhibitory effect to the start-up process existed when effluent recycling was introduced to the reactor system.

### 3.2.2. Methane composition

Methane production during the two start-up phases were measured, and the results are shown in Fig. 5. Methane volume increased from 0.065 to 0.13 L/d, while methane concentration increased from 42 to 62% for 15 d of the first start-up. In the end, a total of 0.013 L $CH_4$/g COD were produced. A previous research on the treatment of paper mill wastewater using an up-flow anaerobic reactor seeded with anaerobic digested sewage sludge shows similar trends; the said study indicates that the highest methane composition at only 62% was produced in stage 2 of the reactor [23].

The methane concentration fluctuated from 57 to 87% during the second start-up/phase1, but the methane volume significantly increased from 0.08 to 0.26 L/d. A total of 0.034 L$CH_4$/g COD were yielded in 15 d. The methane concentration similarly fluctuated between 42 and 86% in the second start-up/

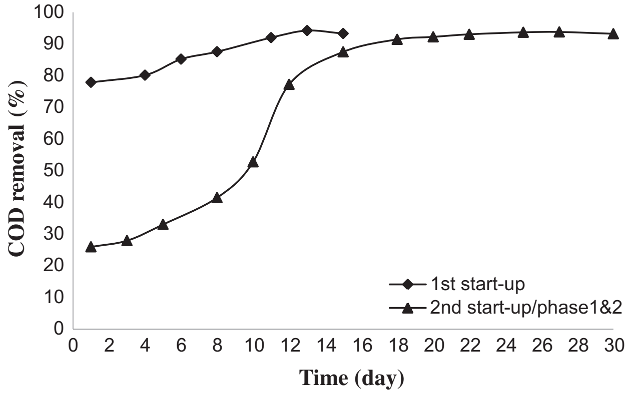

Fig. 4. COD removal efficiency at different start-up technique.

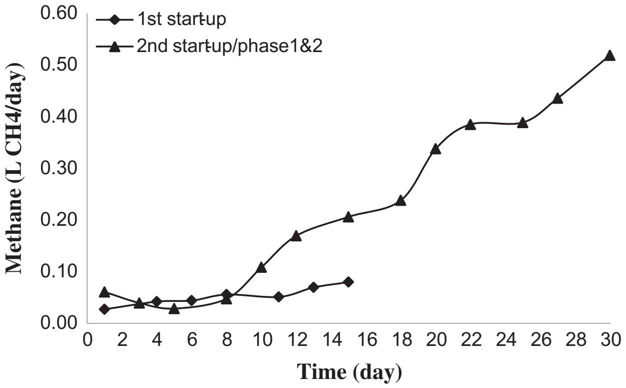

Fig. 5. Methane production at different start-up technique.

phase2, indicating that the methane yield increased because of effluent recycling and reached a maximum value of 0.067 L $CH_4$/g COD. Different results was reported by Grover et al. [8], who studied the black liquor digestion using ABR, whereby they observed that methane yield was 0.096 L $CH_4$/g COD during the start-up at an HRT of five days.

The methane yield obtained is considered low against the theoretical value of 0.35 L $CH_4$/g COD, especially when a high COD removal of up to 90% is achieved. The reason for this finding is probably due to the large amount of removed organic substrates utilized by microorganisms during the start-up phase to generate new biomass cells in the form of g VSS/L, with the rest converted to methane [24]. A relatively slow methane yield can also mean that methane-forming bacteria still do not adapt well to the reactor condition and to RPME feeding.

The observed methane yield refers only to the fraction of COD removed that is effectively converted to methane. However, other COD parts may have been removed through other means, such as solids accumulated in the reactor, part of the VFA adsorbed into the sludge, or precipitation of some compounds within the reactor [25]. In our case, a large part of the influent TS was lignin particles that are resistant to biodegradation; these particles tend to accumulate inside the reactor and are not converted to biogas for a short period of time [16].

Low methane yield in the first start-up can imply that the JSTP is unsuitable for the RPME treatment process unlike the POME. Nevertheless, the second start-up/phase2 proves that effluent recycling has a significant favorable impact on reactor performance. Increasing the OLR by lowering HRTs generally increases biogas production, but our data also illustrate that effluent recycle can augment biogas production. This finding has been proven from previous study where the reactor operations at long HRTs and low effluent recycle ratios can generate biogas with high methane content and yield [26].

### 3.2.3. pH profile

Microbial groups involved in anaerobic degradation have a specific pH region for optimal growth. The desired pH for anaerobic treatment is between 6.5 and 7.6 [27]. Values outside this range can be detrimental to the process, particularly to methanogenesis. Effluent and influent pH levels of the first start-up, second start-up/phase1, and second start-up/phase2 were compared as shown in Fig. 6(a). The temporal reduction in effluent pH was varied in a narrow range for each start-up. The effluent pH level decreased from 7.4 to 6.6 within 10 d and slightly increased for the remaining 5 d during the first start-up. The effluent pH dropped from 7.6 to 6.9 in the second start-up/phase1. Furthermore, effluent recycle did not impair the effluent pH level and reached its steady state value of 6.7. Gradual reduction in effluent pH might be due to the accumulation of VFA resulted by the activities of acidogens. However, effluent pH levels higher than 6.5 indicate that the slower growing methanogens and certain acetogens were not affected by the fast growing acidogens [28].

The influent pH was neutral, and so no alkaline adjustments were used. Despite the slight variations in pH of the feed wastewater, the pH values of the treated effluent were stable at a particular start-up. These results were consistent with the findings of Rongrong et al. [29], who reported that the final effluent remained higher than 6.8 when the influent pH was varied throughout the experimental study. Simultaneously, the effluent pH levels were generally stable (pH > 6.5), which indicates stable performance during the start-up of an anaerobic reactor [17].

To evaluate the characteristic pH levels, the pH profiles of compartments 1 to 5 were measured for the first start-up, second start-up/phase1, and second start-up/phase2, as shown in Fig. 6(b)–(d), respectively. The first start-up and second start-up/phase1 show the same pattern but with different pH levels. The pH level in each compartment decreased in a linear fashion within 10 d and slightly became constant thereafter. The POME stimulated a higher pH level in the reactor in the second start-up/phase1 as compared to the sewage sludge used in the first start-up.

Ran et al. [30] reported that the pH levels in four compartments of ABR decreased gradually in the start-up stage from 4 to 6.4. Relatively high pH values in the front compartments indicate that the MAI-BR configuration encourages the production of intermediate products in the front compartments that are suitable to the methanogenic zone. Reactor design is important for the selection of microbial populations within the system [31].

The pH level was almost constant throughout the reactor during the second start-up/phase2. This condition can be due to the effect of the effluent recycle, which has shown better performance in terms of biogas production. Similar results were also reported by Rongrong et al. [29] whereby the pH levels in a hybrid anaerobic baffled reactor (HABR) of the four compartments remained at a stable level because of the adoption of the effluent recycle. Increasing biogas production and methane content were also associated with the stable and favorable pH value. This finding shows that maintaining a suitable and stable pH

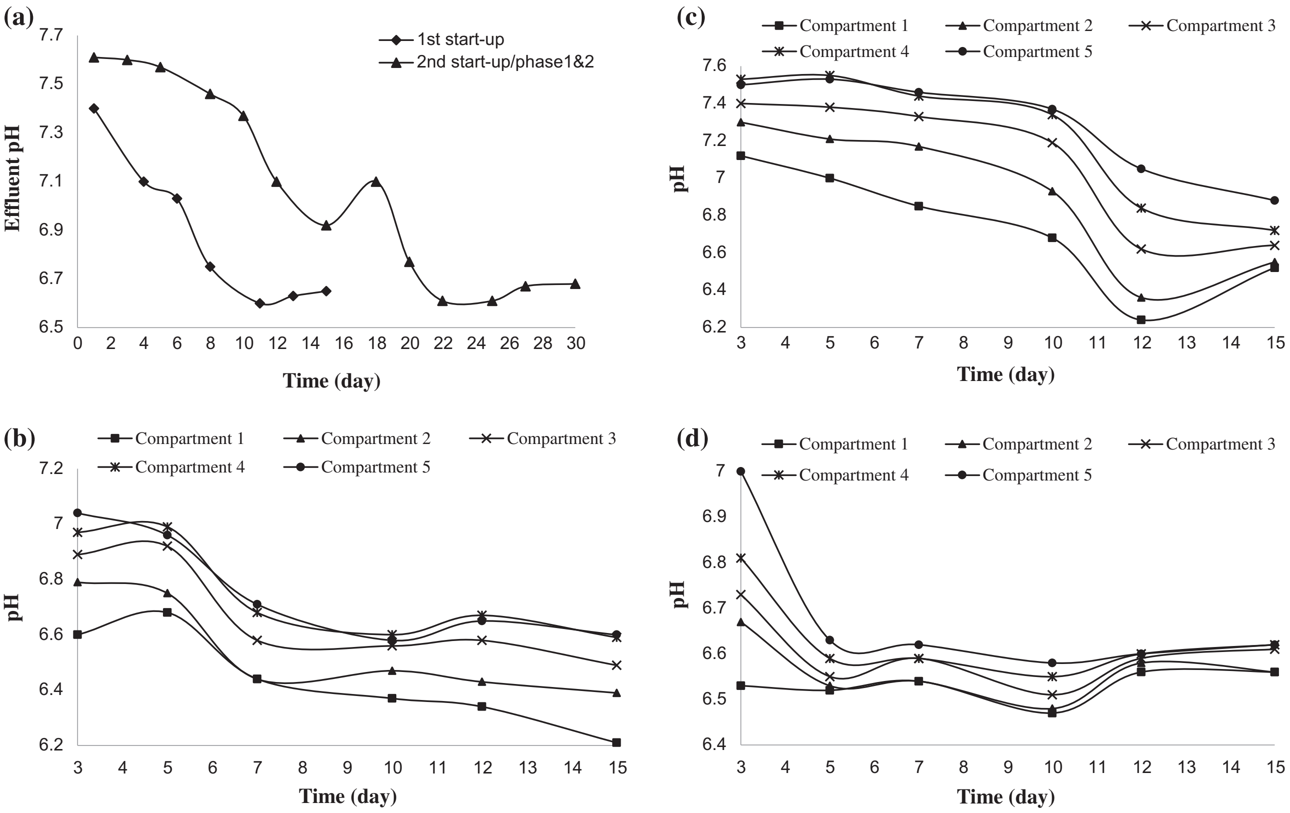

Fig. 6. pH profiles: (a) effluent and influent pH levels at different start-up technique, (b) compartmental pH levels at the first start-up, (c) compartmental pH levels at the second start-up/phase1, and (d) compartmental pH levels at the second start-up/phase2.

within the reactor should be a major priority to ensure efficient methanogenic digestion [32].

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

During the steady state of each start-up, the parameters of COD, BOD, TSS, VSS, fatty acids, alkalinity, pH, tannins, and floc size were tested for the influent, compartments 1 to 5, and effluent. The steady condition was marked by relatively stable effluent COD values with less than 5% variation for each start-up phase [19].

#### 3.3.1. *BOD and COD*

The average COD removal during the steady state were approximately 94%, while the average BOD removal was 93%. A similar trend throughout the reactor was achieved for each start-up in terms of COD and BOD removal. This phenomenon shows that the MAI-BR configuration plays a major role in COD and BOD removal compared with the seeding sludge used. The COD and BOD removal at the compartmental level decreased as RPME passed through the reactor (as shown in Table 3). The COD was less than 100 mg/L in compartment 2, while the BOD was less than 30 mg/L at different start-up condition. Notably, the highest COD and BOD removal occurred in the first compartment, whereas each successive compartment removed only small fractions of the influent COD and BOD. This implies that substrate solubilization takes place at the first compartment of the reactor proposing to be the hydrolysis phase of the system.

The above results on COD and BOD removal are comparable with other study on the treatment of paper mill wastewater using stage anaerobic reactor [23]. Chelliapan et al. [23] reported that approximately 88% of the COD was removed during the acclimatization period. They also noted that most of the COD was removed in stage 1, whereas stages 2, 3, and 4 showed relatively minor contributions (less than 10%) to the total COD removal. The removal efficiencies were lower when the COD concentration decreased in the subsequent compartments. This might be due to the reduction in substrate utilization rate of the microorganisms in the preceding compartment

Table 3
The modified anaerobic inclining-baffled reactor performance at different start-up technique under a steady-state condition

|  | COD (mg/L) |  |  | BOD (mg/L) |  |  | VSS (mg/L) |  |  | Fatty acids (mg/L) |  |  | Alkalinity (mg $CaCO_3$/L) |  |  | pH |  |  | Floc size (μm) |  |  | Lignin (mg/L) |  |  |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Start-up | 1st | 2nd/1 | 2nd/2 | 1st | 2nd/1 | 2nd/2 | 1st | 2nd/1 | 2nd/2 | 1st | 2nd/1 | 2nd/2 | 1st | 2nd/1 | 2nd/2 | 1st | 2nd/1 | 2nd/2 | 1st | 2nd/1 | 2nd/2 | 1st | 2nd/1 | 2nd/2 |
| Influent | 952 | 1,032 | 949 | 475 | 505 | 453 | 1,160 | 910 | 1,024 | 134 | 124 | 75 | 275 | 135 | 180 | 7.94 | 6.9 | 7.75 | 199 | 201 | 179 | 13 | 15 | 14 |
| Compartment 1 | 137 | 65 | 76 | 54 | 13 | 21 | 14,710 | 23,360 | 13,373 | 18 | 84 | 30 | 325 | 195 | 220 | 6.21 | 6.52 | 6.57 | 151 | 93 | 104 | 10 | 15 | 9 |
| Compartment 2 | 58 | 77 | 64 | 31 | 20 | 20 | 14,810 | 16,127 | 10,660 | 17 | 85 | 9 | 380 | 328 | 215 | 6.39 | 6.55 | 6.56 | 112 | 87 | 115 | 10 | 15 | 7 |
| Compartment 3 | 64 | 94 | 74 | 34 | 24 | 28 | 13,250 | 11,657 | 2,240 | 16 | 71 | 6 | 500 | 593 | 210 | 6.49 | 6.64 | 6.61 | 115 | 71 | 88 | 8 | 23 | 7 |
| Compartment 4 | 60 | 109 | 60 | 38 | 30 | 26 | 13,390 | 5,460 | 1,293 | 13 | 43 | 5 | 630 | 935 | 220 | 6.59 | 6.72 | 6.62 | 125 | 64 | 87 | 8 | 28 | 8 |
| Compartment 5 | 62 | 118 | 60 | 36 | 37 | 29 | 14,490 | 2,460 | 447 | 7 | 47 | 4 | 640 | 1,375 | 210 | 6.6 | 6.88 | 6.61 | 126 | 53 | 64 | 7 | 30 | 7 |
| Effluent | 61 | 125 | 64 | 34 | 39 | 32 | 160 | 164 | 116 | 6 | 44 | 3 | 650 | 1,370 | 210 | 6.63 | 6.92 | 6.67 | 25 | 56 | 67 | 7 | 35 | 7 |

occurred, which led to low removal efficiency. This phenomenon can be properly supported by the bacterial kinetics when low growth rate is caused by low substrate concentration [26].

### 3.3.2. pH level

Table 3 shows pH level profiles in every part of the reactor for each start-up phase. All three start-up phases show a favorable pH level throughout the reactor, indicating a stable reactor performance during the study of seed source effect and effluent recycle. The pH drop was observed in compartment 1 and steadily increased as the wastewater moved inward to the subsequent compartments in the compartmental level of the first start-up and second start-up/phase1. The range of pH levels within the reactor were 6.21–6.6 and 6.52–6.9 for the first start-up and second start-up/phase1, respectively. Reduction in pH levels at front compartments and its increment at successive compartments indicate appropriate placement of acidogens and methanogens in the system. Similar results were also reported elsewhere, where the pH in the first compartment dropped during the anaerobic process, whereas other compartments were the least affected [18,32].

In the case of the second start-up/phase2, the effluent recycle changed the previous pattern and improved the pH level up to 6.7 in the first compartments. This indicate that effluent recycle has provided more methanogens to the first compartment, hence maintaining the desired pH. This confirms the ability of the effluent recycle to increase the front pH of the system. These findings are also consistent with the results of other researcher [26], who reported that the effluent recycle returns part of the alkalinity left in the effluent back to the system. Thus, the returned effluent plays an important role in maintaining the pH balance of the system that allows effective anaerobic digestion.

As a final point, the effluent pH remained higher than 6.5 throughout the experimental study. The stable effluent pH values imply the effective consumption of VFA by methanogens. Thus, the remarkable increase in biogas production and methane content (% $CH_4$) is associated with the stability in pH level [32]. Within the acidogenic dominant zone of an anaerobic reactor, the low pH levels play a vital role in microbial selection [33]. Thus, the souring of the reactor did not occur during the entire start-up operation period. The pH value and alkalinity are important factors that measure the stability of the anaerobic reactor.

### 3.3.3. Alkalinity and fatty acids

Low values of alkalinity imply an impending reactor failure. Many studies have adjusted the influent alkalinity to stabilize the anaerobic process [28,34]. In this study, no influent adjustment was used and the MAI-BR performance remained stable. The results showed that during the start-up period (i.e. the time of acclimatization at new conditions), alkalinity levels were lower at the first compartment and then increased in the subsequent compartments, as shown in Table 3. The average alkalinity levels in the five compartments were 495, 685, and 215 mg/L for the first start-up, second start-up/phase1, and second start-up/phase2, respectively. Reduction in alkalinity level in the Compartment 1 could be attributed to the substrate hydrolysis and acid production resulted by the acidogens microbes, whereas an increment in the successive compartment is due to the generation of bicarbonate and carbonate by acetogens microbes.

All five compartments also underwent significant variations of alkalinity and VFA during the three start-up phases, which were concurrent with the fluctuation in $CH_4$ production. Similar observations in the ABR treating soybean protein processing wastewater were reported by Zhu et al. [35], whereby alkalinity level in the first compartment was found to drop to 350 mg/L. In another study on the treatment of low-strength wastewater using ABR [36], the alkalinity increased from 360 to 435 mg/L as the wastewater moved from the first to the fourth compartment. The researchers also observed that the increase in alkalinity was correlated with the increase in pH level and the decrease in VFA.

High alkalinity levels at a start-up phase reveal the stability of the reactor toward toxic matter accumulations and its resistance to system failure. Low alkalinity at the first compartment is generally significant and can be alleviated using effluent recycle. The recycled stream was originally believed to provide additional alkalinity for a pH control purpose and possibly returned part of the alkalinity left in the effluent back to the system. In the study reported by Saritpongteeraka and Chaiprapat [26] on ABR treating high-sulfate wastewater, the addition of a recycle stream could return part of the alkalinity left in the effluent back to the system. Apart from that, the ratio of VFA to total alkalinity can also be used as a measure of stability of an anaerobic process. This ratio indicates a balance between acidogenesis and methanogenesis within the reactor, and it should be maintained below 0.5 for proper anaerobic functioning [37]. In this study, the compartmental ratios varied in ranges of 0.06–0.01, 0.43–0.03, and 0.14–0.02 at the first start-up, second start-up/phase1, and second start-up/phase2, respectively. Based on these obtained ratios, all start-up phases showed excellent system performance.

The VFA variation profiles in the MAI-BR for each start-up phase are shown in Table 3. The VFA values decreased longitudinally down the reactor (near the effluent point). This might be due to the breakdown of complex fatty acids after hydrolysis from compartment 1 into simpler short chain VFAs. The highest VFA concentrations were found in the first compartment, with average values of 134, 124, and 75 mg/L for the first start-up, second start-up/phase1, and second start-up/phase2, respectively. For comparison, Gopala Krishna et al. [38] observed the formation of VFA (53–85 mg/L) in the first compartment of ABR because of acidogenesis and acetogenesis.

From VFA data, it demonstrates that hydrolysis and acidogenesis were the main biochemical activities occurred in the first compartment. Methanogenesis also appears to be dominant in the last few compartments. These observations suggested that the ABR system promotes a systematic selection in the different compartments in a manner that results in phase separation. Torabian et al. [19] also reported that the total VFA concentration decreased longitudinally down the reactor from compartment 2 while treating low-strength industrial wastewater using ABR.

Compartmental-wise VFA production profiles suggested that only the biomass in the first compartment are stressed (increased VFA concentration) at lower OLRs of 0.2 g/L d, whereas the biomass in more compartments can be stressed at higher OLRs. However, the five compartments collectively functioned to stabilize the MAI-BR. The first compartment showed signs of stress (e.g. low pH and high VFAs) at a certain start-up, but this effect disappeared in the last compartments; if the last compartments were stressed, the total performance of the VFA consumption would be observed, which could affect the methanogenesis in the next compartments [39].

The accumulation of fatty acids was insignificant in the second start-up/phase2, which did not affect the methanogenesis that occurred in the reactor. This condition can be due to the low activity of hydrolytic and acidogenic bacteria compared with methanogenic bacteria. However, the VFA level in the next compartments significantly decreased and the pH was stable. This findings indicates that methanogenic bacteria became more active in the first compartment because of the improvement in pH level provided by the effluent recycle. This phenomenon demonstrates that different microenvironments select the dominant bacteria population. In addition, the varying VFA and alkalinity along the MAI-BR reflect the success of reactor start-up.

### 3.3.4. *Suspended solids*

The success of anaerobic reactor performance is related to its ability to maintain a high biomass inventory, especially during start-up. Determining if the granular biomass can withstand settling under determined start-up operation conditions is important because this can eventually result in biomass washout and reactor failure. Table 3 shows the VSS variation in each compartment with the variation in start-up techniques. The TSS concentration of the influent ranged from 1,092 to 1,430 mg/L (data not presented in Table 3) with 81–90% were in the VSS form. Among all compartments, the first compartment generally had the highest VSS content, which then significantly decreases at the rear end compartment. The higher VSS concentration in compartment 1 is because only the soluble part of substrate gets transferred to the successive compartments and about 21 to 48% of the VSS are retained back at compartment 1. Furthermore, this could be due to the slow growth rate of the acetogenic and methanogenic populations as compared to the hydrolytic and acidogenic microbes. The results are consistent with those reported by Zhang et al. [40], who obtained high sludge load in the first two compartments of the ABR that treats domestic wastewater.

Furthermore, the VSS concentrations in the treated effluent were 160, 164, and 116 mg/L for first start-up, second start-up/phase1, and second start-up/phase2, respectively. The VSS concentrations in the treated effluent were not affected by the varying VSS concentrations in the influent wastewater at a particular start-up. The first start-up showed that the mixture of POME seed sludge and JSTP has a higher VSS content than the second start-up/phase1, which was seeded using the POME seed sludge. However, this result does not correspond to the better performance during the second start-up. Nevertheless, the VSS content may not be the only factor for an efficient anaerobic process that exist in the system. This results concluded that the system design facilitates low biomass washout during the start-up and offers very high specific reaction rate. To prevent the loss of biomass, the addition of packing materials could help to stop the escaped biomass subsequently increasing the performance.

Furthermore, the effluent recycle in the second start-up/phase2 did not affect the solid washout, and a slight reduction in VSS concentration was noticed. The biomass washout can be indicated by the VSS/TSS ratio and granule size distribution in the sludge. The importance of this ratio is that it defines the degradation of inert solids in the reactor by considering the difference between TSS and VSS in the sludge and the difference in the influent wastewater [41]. The VSS/TSS ratio of 64–71% observed in the MAI-BR sludge was necessary and sufficient to control the biomass washout. These results showed that a relevant fraction of the incoming inert particulate material did not accumulate in the reactor possibly because of hydrolysis or enzymatic solubilization.

### *3.3.5. Lignin and floc size*

Lignin is generally inaccessible to most microorganisms because of its heteropolymeric structure of phenylpropanoid subunits, which build a relatively stable biopolymer [42]. Table 3 shows the variation in lignin content in each compartment with the variation in start-up techniques. The RPME was diluted four times, and the influent lignin average content was 14 mg/L. Lignin concentration in the reactor was in the form of dissolved lignin which was affected by the lignin content in the RPME sludge, especially at the start-up phase. For example, POME sludge was used for seeding in the second start-up/phase1 had 58 mg/L of lignin [16]. A 50% reduction in the lignin content was observed in the second start-up/phase2 of the (MAI-BR) start-up. Similar trend reported elsewhere, whereby about 51% of the lignin was removed from the NSSC pulping effluent using a UASB reactor [43]. Table 3 shows the floc size of each compartment, influent, and effluent. The influent floc size initially ranged from 179 to 201 μm. After 15 d, the average floc sizes in the reactor were 126 and 74 μm for the first start-up and second start-up/phase1, respectively. The first start-up shows a larger floc size as compared to the second start-up/phase1 due to different seeding sludge. As a continuation of the second start-up/phase1, the floc size of the second start-up/phase2 increased to 94 μm in 30 d. The effluent recycling did not affect the floc size. Compartment 1 generally had the largest floc size and gradually decreased. The dip toward the rear could be attributed to the low substrate levels that result in low bacterial growth and smaller flocs, although this explanation is still unproven [44].

## 4. Conclusion

The seeding sludge microbial activities test is a sufficient indicator of seeding microbial activities. The findings match the seed sludge suitability during the start-up of a continuous system and support the anaerobic microorganism activity in a batch scale. The present study shows that the MAI-BR start-up was successfully performed and significantly improved within 30 d. At the same time, the second start-up/phase1 proved that POME exhibited better performance compared with the first start-up, which was seeded using a mixture of POME and JSTP sludge. As a continuation of the second start-up/phase1, effluent recycling is an important factor that leads to a successful and efficient start-up operation, especially for high solid-content wastewater. This process showed improvement in pH level, biogas volume, methane content, VSS content, floc size, and lignin removal. Therefore, the reactor can continue running with effluent recycling using the same operation conditions in the second start-up/phase1 to study the HRT and OLR effects.

## Acknowledgments

This work was financial supported by Universiti Sains Malaysia (RU-I Grant A/C. 1001/PJKIMIA/814148). The authors wish to thank Muda Recycled Paper Mill Sdn Bhd for their support.

## Abbreviations

AD — anaerobic digestion
ABR — anaerobic baffled reactor
MAI-BR — modified anaerobic inclining-baffled reactor
RPME — recycled paper mill effluent
POME — palm oil mill effluent
JSTP — Jelutong sewage treatment plant
ISR — substrate to inoculum ratio
OLR — organic loading rate (g/L d)
HRT — hydraulic retention time (d)

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