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# Temperature analysis of a novel MAIB reactor during the treatment of wastewater from recycled paper mill

Haider M. Zwain[a,b], Ahmed Samir Naje[a], Mohammadtaghi Vakili[c] and Irvan Dahlan[d,e,*]

[a] College of Water Resources Engineering, Al-Qasim Green University, Al-Qasim Province, Babylon, Iraq

[b] Department of Civil and Architectural Engineering, College of Engineering, Sultan Qaboos University, P.O. Box 33, Al-Khodh, 123 Muscat, Oman

[c] Green Intelligence Environmental School, Yangtze Normal University, Chongqing 408100, China

[d] School of Chemical Engineering, Universiti Sains Malaysia, Engineering Campus, Seri Ampangan, 14300 Nibong Tebal, Penang, Malaysia

[e] Solid Waste Management Cluster, Science and Engineering Research Centre, Universiti Sains Malaysia, Engineering Campus, Seri Ampangan, 14300 Nibong Tebal, Penang, Malaysia

*Corresponding author. E-mail: chirvan@usm.my

## Abstract

Anaerobic digestion (AD) is an essential technology for wastewater management, resource recovery and biogas production, and it is considered as an efficient and reliable treatment method for many wastewaters. Operating parameters have been shown to directly affect the stability and treatment performance of AD, especially temperature. For 180 days, the AD of recycled paper mill wastewater (RPMW) was carried out in a modified anaerobic inclining-baffled (MAIB) reactor under various temperature conditions, i.e. 29 °C (low mesophilic), 37 °C (mesophilic) and 55 °C (thermophilic). It was found that total COD removal of 94, 96 and 76%, and methane yields of 0.125, 0.196 and 0.256 L $CH_4$/g $COD_{removed}$ were attained at temperatures of 29, 37 and 55 °C, respectively. Throughout the three transition periods, the pH level in the MAIB reactor fluctuated slightly within the range of 5.8–6.5 without affecting the system stability. The results concluded that thermophilic condition strongly influenced the MAIB reactor performance, leading to lower COD removal, higher methane yield and gradually recovered pH level.

**Key words:** anaerobic digestion (AD), mesophilic temperature, modified anaerobic inclining-baffled (MAIB) reactor, recycled paper mill wastewater (RPMW), thermophilic temperature

## Highlights

- Novel modified anaerobic inclining-baffled (MAIB) reactor, which combines the development of attached and settled microorganisms, was operated for the treatment of recycled paper mill wastewater (RPMW).
- The operational temperature intensive analysis of MAIB was investigated at thermophilic and mesophilic conditions.
- The system compartmental characteristics were also analyzed and discussed.

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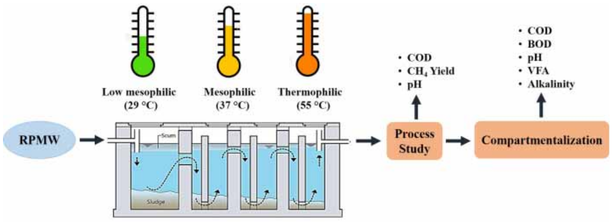
## Graphical Abstract




## INTRODUCTION

One of the most important industries in the world, especially in Malaysia, is the recycled paper industry. In Malaysia there are actually 20 paper mills, 18 of which use raw materials of 100% recycled paper. From this recycled paper industry, recycled paper mill wastewater (RPMW) is being generated. RPMW is categorized as a complex wastewater due to significant amounts of suspended solids, BOD, COD, lignin, ammonia, VFA and biodegradable organics (Zwain *et al.* 2016a). Therefore, RPMW needs to be treated according to permissible limits before release into a municipal sewer system or receiving streams.

Due to high amounts of readily biodegradable materials, RPMW could be anaerobically treated with different combinations of biological systems (Cai *et al.* 2019). Anaerobic digestion (AD) is one of the most appropriate and efficient methods that have been used to treat industrial and municipal wastewater, due to its potential to produce methane and a semi-stabilized digestate, which can be further processed as a substitute for inorganic fertilizers (Chatterjee & Mazumder 2019). However, for the effective utilization of AD in industrial wastewater treatment, a successful development of high-rate anaerobic reactors is required.

Through maintaining the biomass in the reactor for longer time, a high conversion wastewater biodegradation can be obtained. In this regard, the anaerobic baffled reactor (ABR), as a modification of the up-flow anaerobic sludge blanket reactor, was found to be an effective system for various wastewater treatment. It is a staged reactor that promotes biomass retention within the system by driving water flows up and down across several compartments (up to 8 compartments) (Zhu *et al.* 2015). Subsequently, the efficacy of anaerobic digestion is defined by the microorganism behavior and quantity, which are influenced by wastewater composition, system configuration and operation condition, and the microbial content formed in the anaerobic reactor (Zwain *et al.* 2019b).

A key aspect influencing the AD process is the operating temperature (Khalekuzzaman *et al.* 2018). Increasing or decreasing temperatures may affect the biological reaction rate or digestion performance within the ABR (Ali *et al.* 2019; Chang *et al.* 2020). Reduction in temperature is extremely negative for the anaerobic microorganisms' metabolic behavior, whereas increase in temperature, up to an optimum level, enhanced the microbial activity (Zamanzadeh *et al.* 2013). Regarding the RPMW, it is usually discharged at temperatures around 35–50 °C, which enables mesophilic or thermophilic anaerobic condition to be operated without inhibition. The effect of seasonal temperature variations on anaerobic treatment has been widely studied (Li *et al.* 2019); thus, wide temperature variation may also highly affect the anaerobic digestion of RPMW (Zwain *et al.* 2019a).

In this regards, thermophilic anaerobic condition was found to be more effective than the mesophilic in term of the specific methane yield, generated energy, effluent quality and process stability as compared to mesophilic condition (Ramakrishnan & Surampalli 2013). However, limited studies have investigated the temperature changes on the anaerobically RPMW treatment. Apart from that, thermophilic anaerobic wastewater treatment is hardly applied, due to many inconsistent and occasionally disappointing results being obtained by many researchers (Zwain *et al.* 2019a; Chen *et al.* 2020; Chen *et al.* 2021). Therefore, the current study aims to study the influence of changing temperature during RPMW treatment by using

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a modified anaerobic inclining-baffled (MAIB) reactor. For three transition periods of 60 days each, the reactor system was operated at 29 °C (low mesophilic), 37 °C (mesophilic) and 55 °C (thermophilic).

## METHODOLOGY

### Seed and RPMW source

The RPMW was obtained from one of the paper mills located in Simpang Ampat, Penang, Malaysia. The RPMW samples were stored in cooling room at 4 °C in order to avoid chemical and biological reactions. They were then warmed up to surrounding temperature (29 $\pm$ 2 °C) prior to being streamed to the reactor. The collected RPMW had a BOD/COD ratio of 0.49, total COD concentration of 3,812 mg/L, and VSS concentration of 1,967 mg/L. The system was seeded with flocculant anaerobic sludge collected from an anaerobic pond treatment system treating palm oil mill effluent (POME), located in Malpom Palm Industries Bhd, Penang, Malaysia. It had a total COD concentration of 32,137 mg/L, TSS concentration of 4,135 mg/L, and VSS concentration of 3,535 mg/L. Further details on the optimization of the start-up process, including inoculum sources and activity test, inoculum characterization, and inoculum loading have been previously described (Zwain *et al.* 2016a, 2016b).

### Reactor design and configuration

The reactor used is a MAIB laboratory-scale reactor, which was constructed using polypropylene plastic with an effective volume of 35 L and had dimensions (length $\times$ height $\times$ width) of 80 cm $\times$ 30 cm $\times$ 15 cm. The reactor had five compartments, divided by a modified vertical baffle, where the baffle's lower part bent to route the flow into the up-flow compartments. To maintain the reactor temperature, a water jacket was attached to the reactor. The reactor layout is shown in Figure 1, and further details of the system configuration have been previously illustrated (Zwain *et al.* 2017).

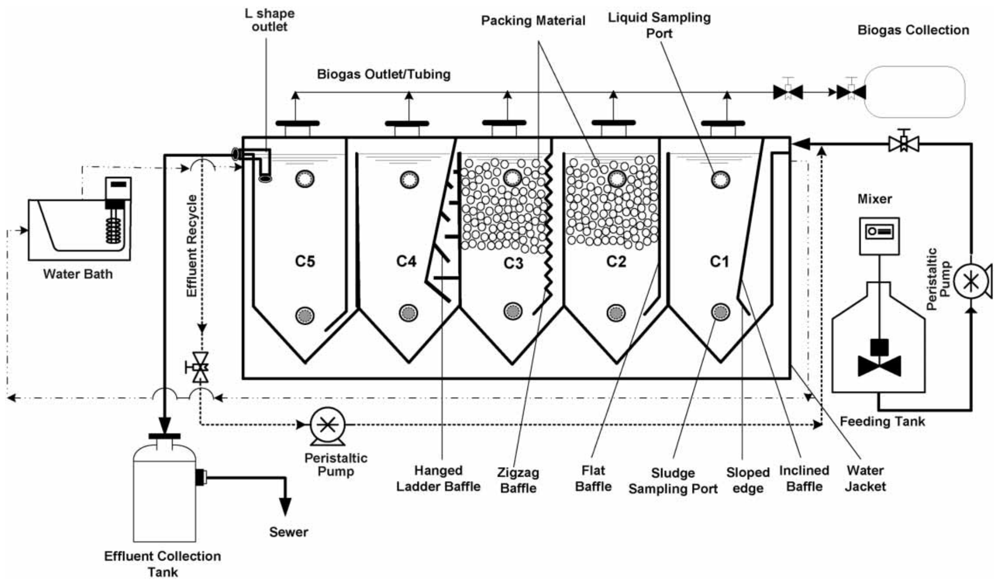


**Figure 1** | The MAIB reactor layout showing the system configuration, sampling points, biogas collection and feeding tank.

### Experimental method

The detail of operational conditions of the MAIB, including the acclimation of the reactor microorganisms and the control of pH has been previously described (Zwain *et al.* 2017). During 180 days, the

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temperature's influence on the treatment of RPMW was investigated at 29 °C (low mesophilic), 37 °C (mesophilic) and 55 °C (thermophilic) conditions, as shown in Table 1. Initially, the reactor was operated at low mesophilic condition followed by an increase in temperature to mesophilic condition, then increased to thermophilic condition. To stabilize the MAIB reactor at each temperature transition period, the reactor was operated in three phases. In the first phase, temperature acclimation was carried out with gradual increase in temperature by 2 °C daily until the desired temperature was reached. During the second phase, the MAIB reactor reached the desired temperature and was continuously run with an OLR of 1.25 g/L.d at constant feed with 2,500 mg/L of total COD concentration and 2 days' HRT. During the last phase, the reactor's steady state performance was evaluated for compartmental level. The experimental transition period of 60 days was found sufficient for the reactor to reach steady state.

**Table 1** | The MAIB reactor conditions at different temperature, named low mesophilic (29 °C), mesophilic (37 °C) and thermophilic (55 °C)

| Period | Temperature conditions | OLR (g COD/L day) |
|---|---|---|
| Day 0–60 | Low mesophilic (29 °C) | 1.25 |
| Day 61–120 | Mesophilic (37 °C) | 1.25 |
| Day 121–180 | Thermophilic (55 °C) | 1.25 |

### Analytical methods

COD removal, biogas generation, and pH level were monitored during the continuous feeding. Throughout the operational period, samples from the in flow, out flow and biogas were analyzed every two days onward until a steady-state condition was achieved. The BOD, total COD, pH, VFA and alkalinity of the reactor compartmental were observed during this steady-state condition. The VFA and total COD were tested using a spectrophotometer (DR-2800 model). The concentration of methane ($CH_4$) was analyzed using a Shimadzu GC-FID with a Propack N column. The pH level was analyzed using a pH meter (CyberScan pH 510 model). Following the standard methods (APHA *et al.* 2012), the concentration of alkalinity and BOD were measured.

## RESULTS AND DISCUSSION

### MAIB reactor performance

#### Influence of temperature changes on COD removal efficiency

Figure 2 illustrates the total COD removal profiles for different temperatures of the MAIB reactor system. The maximum total COD removal efficiencies were obtained up to 94, 92 and 76% at 29 °C (low mesophilic), 37 °C (mesophilic) and 55 °C (thermophilic), respectively. A very high COD removal cannot be explained by only the biodegradation process, because it is not theoretically possible to biodegrade >90% COD for this RPMW. According to the BOD/COD ratio, the total COD can be fractioned into about 49% of biodegradable COD removal by anaerobic digestion and about 51% of non-biodegradable COD removal by physical settlement. The MAIB reactor system contains baffles that promote phase separation, which led to a combined effect of both of biological and physical processes in one system.

In general, the COD removal efficiency gradually increased for all temperatures. As shown in Figure 2, the best reactor performance was achieved at 37 and 29 °C. In an anaerobic membrane reactor treating municipal wastewater, Martinez-Sosa *et al.* (2011) reported that up to 90% COD removal efficiencies were achieved under psychrophilic and mesophilic temperature ranges. Another study on

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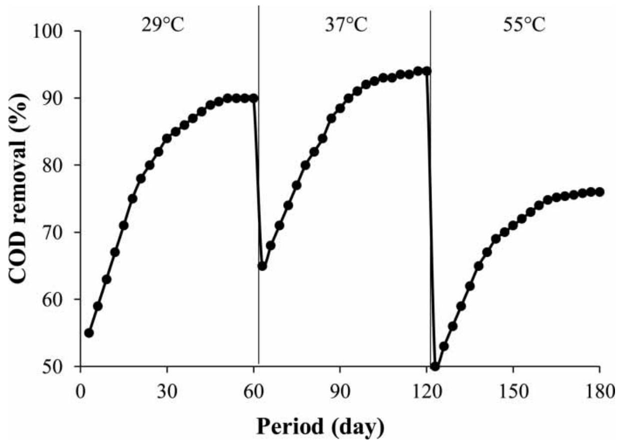


**Figure 2** | COD removal profiles at low mesophilic (29 °C), mesophilic (37 °C) and thermophilic (55 °C) condition.

the treatment of synthetic purified terephthalic acid wastewater conducted by Li *et al.* (2014) showed that high COD removal of 92% could be achieved at mesophilic condition (37 °C) compared to 61% at thermophilic condition (52 °C).

However, in this study, when the RPMW was treated in thermophilic conditions, the COD removal decreased. A decrease in removal of COD over a short time period could be due to several reasons. Theoretically, at thermophilic temperature, reaction rates are higher than at mesophilic temperature. However, the microorganism growth rate is believed to be concurrently affected by protein denaturation at thermophilic temperatures. Proteins are one of the main constituents of microorganisms and can irreversibly denature at high temperature (Zwain *et al.* 2019a). Thermophilic denaturation of proteins may underlie some observed effects such as reduction in the microbial activities, resulting in the reduction COD removal.

**Influence of temperature changes on methane production**

Methane production and its potential usage as an alternative source of energy is an interesting advantage to anaerobic treatment of wastewater. Methane yield (L $CH_4$/g $COD_{removed}$) and methane content (%) were measured during this study and the results are shown in Figure 3. During the low mesophilic and mesophilic conditions, the methane content was slightly fluctuating around 60–79%. When the temperature was shifted to thermophilic condition, the methane content was remarkably decreased to 51%, then recovered to 76% at the end of this transition period. Likewise, Lv *et al.* (2019) stated that, due to a sudden rise in temperature, equilibrium between fermenting and methanogenic bacteria could be reversed and eventually organic acid will be accumulated, contributing to decreased biogas production.

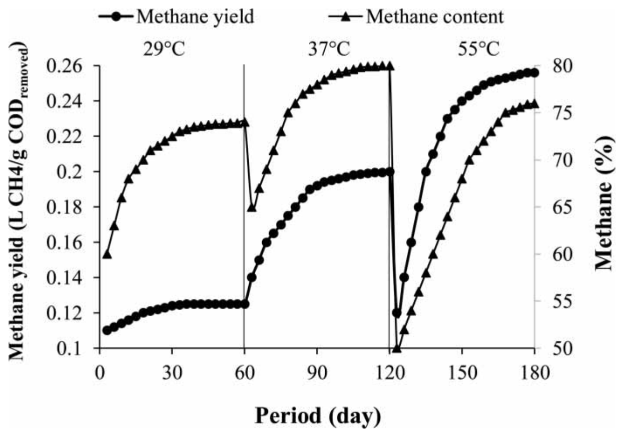


**Figure 3** | Methane yield (L $CH_4$/g $COD_{removed}$) and methane content (%) under low mesophilic (29 °C), mesophilic (37 °C) and thermophilic (55 °C) conditions.

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In the same way, an increase in methane yield was noticed when the temperature was changed from 29 to 37 °C, and it was more evident with temperature increment from 37 to 55 °C. At the steady state condition of each transition period, the methane yields were highest and reached 0.13, 0.2 and 0.256 L $CH_4$/g $COD_{removed}$ at 29 °C (low mesophilic), 37 °C (mesophilic) and 55 °C (thermophilic), respectively. The $CH_4$ yield is calculated based on the produced $CH_4$ per COD removed (L $CH_4$/g $COD_{removed}$) in the reactor, so it depends on both the methane production and COD removal, not only COD removal. Hence, at constant methane production, higher $CH_4$ yield under thermophilic conditions is a result of high conversion of the small COD faction (low COD removal efficiency) available in the system.

The $CH_4$ yield would drop if both methane production and COD removal efficiency dropped. Similarly, in anaerobic filters treating paper mill wastewater, Yilmaz *et al.* (2008) observed that thermophilic conditions produced higher methane yield of 0.291 L $CH_4$/g $COD_{removed}$ compared to 0.274 L $CH_4$/g $COD_{removed}$ in mesophilic conditions. Due to higher substrate digestion rate, thermophilic reactors were found to generate more biogas than mesophilic reactors (Jeong *et al.* 2014). In this study, the methane yield was less than the theoretical methane yield rate of 0.35 L $CH_4$/g $COD_{removed}$. This might be due to the complex RPMW characteristics and the presence of methanogen inhibiters such as VFA.

### Influence of temperature changes on pH level

The pH level is the key factor during the continuous operation of an anaerobic digester. In an anaerobic system, the optimum pH condition for anaerobic microbial activity is neutral pH (Shah *et al.* 2014). Figure 4 illustrates that RPMW had a relatively neutral effluent pH in the range of 6.5–7.3, thus it was suitable for anaerobic digestion without any further alkaline adjustment, and effluent pH level behaved appropriately in fluctuation regarding temperature change. Within each transition period, the fluctuation of effluent pH levels was about 6.2–6.5 for all temperatures. There was a pH decrease after every change in temperature, indicating the proper placement of acidogenesis and methanogenesis. Similarly, Lv *et al.* (2019) reported that changes in pH levels were probably due to anaerobic biomass adaptation and microbial rearrangements to the temperature shock. A drop in pH level in thermophilic conditions was reflected by a reduction of methane production, then it recovered when the pH increased.

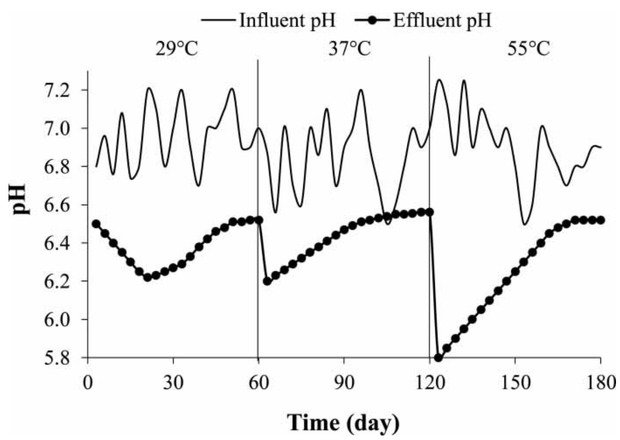


**Figure 4** | Effluent and influent pH levels in low mesophilic (29 °C), mesophilic (37 °C) and thermophilic (55 °C) conditions.

The mesophilic condition has resulted in more stable effluent pH compared to low mesophilic and thermophilic temperatures, whereas the methane yield was gradually increased. This might be due to balanced activity of acidogenic bacteria and methanogenesis in mesophilic condition. The pH level reduction is due to the fermentation of COD to organic acids by acidogenesis. Thereafter, the

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methanogenesis will convert the organic acids to methane gas, resulting in the reduction of VFA and increase in the pH level in the system. The rate of substrate conversion and the activity of microorganisms are highly influenced by change in temperature. Therefore, fluctuation in the pH level was observed throughout the study. These results can be compared with those reported by Ramakrishnan & Surampalli (2013), who reported that the pH in mesophilic conditions was generally higher than in thermophilic conditions. In this current study, a temporal increase in pH level in the rector revealed that the fatty and amino acids dissolved in RPMW had been well converted into intermediate products, and later into carbon dioxide and methane, resulting in an increased pH level in the reactor. This was also supported by Jeong *et al.* (2014), who observed similar results.

## Reactor performance in compartmental process

### Compartmental characteristics of COD and BOD

At steady state, the compartmental characteristic is an additional crucial factor to assess the performance of a multi-stage reactor. Figures 5 and 6 show the compartment profiles of COD and BOD at different temperatures, respectively. The system compartmentalization was reflected by different abiotic parameters in the five compartments. In Compartment 1, the COD concentration decreased significantly with an increase in temperature. In the thermophilic condition, the COD concentration had a better distribution over the reactor because organic matter had become more soluble/dissolved at high temperature. For instance, the COD concentration increased to the rear of the reactor in thermophilic conditions compared to mesophilic conditions. Increase in the COD concentration might be due to solubilization/hydrolysis of organic molecules at higher temperature. This was also supported by Li *et al.* (2015), who stated that organic substrates' hydrolysis into micro-molecular organic acids was easier at high temperature. In this study, thermophilic temperature has improved the RPMW hydrolysis, leading to augmented soluble carbohydrate and protein concentrations. Due to higher activities of fermentative bacteria at 55 °C, relatively sufficient soluble substrates were efficiently converted into VFAs, compared to mesophilic temperatures.

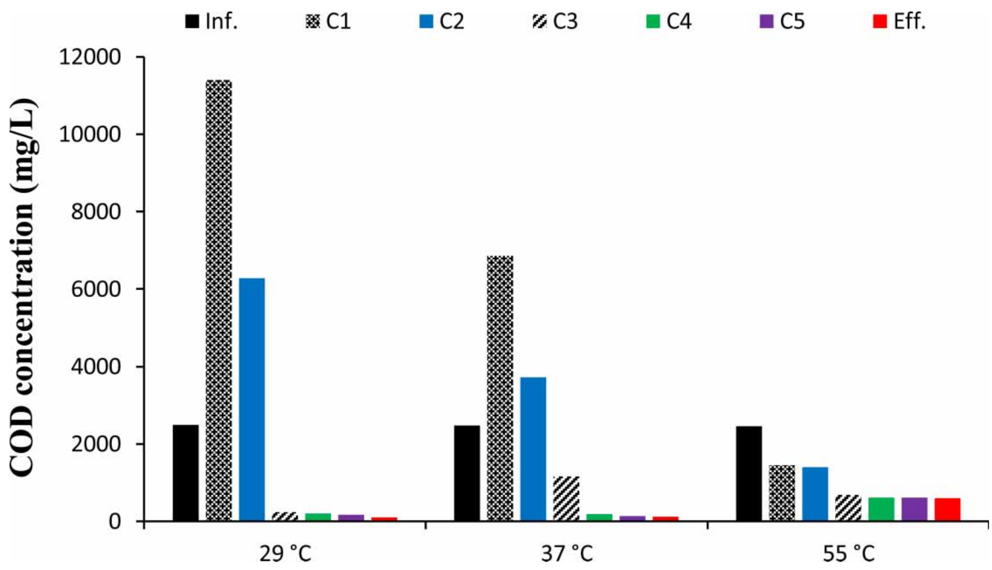


**Figure 5** | Characterization of COD according to MAIB reactor compartments during steady state at different temperature.

Another observation was noticed, where the effluent COD concentration in the mesophilic conditions was much less compared to the thermophilic condition. This might be due to low solubility of non- biodegradable COD at low temperature, led to physical removal of COD by the settlement

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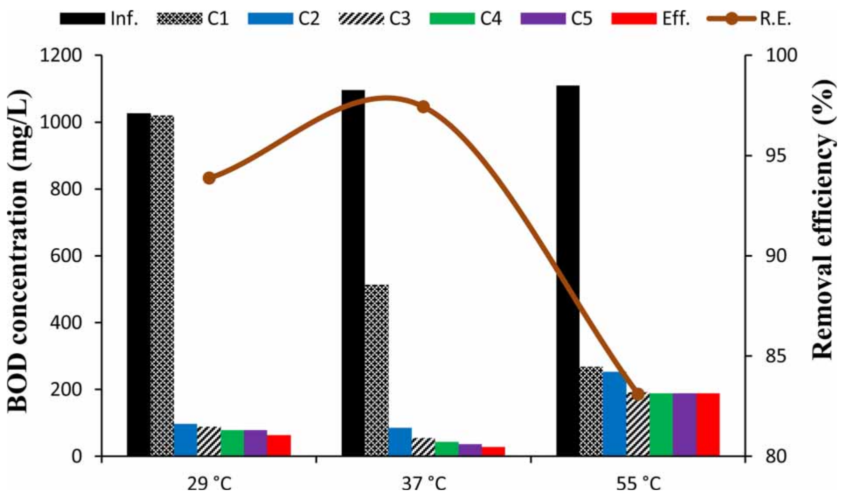


**Figure 6** | Characterization of BOD according to MAIB reactor compartments during steady state at different temperature.

of organic matter in the reactor, especially in the initial compartments. Accordingly, a substrate BOD/COD ratio of 49% seems unreasonable that the COD removal is almost as high as the BOD removal. High COD removal can be explained by biodegradation of BOD and physical accumulation of COD in the reactor, which played a big role in the removal mechanism. A similar trend was also reported by Ayaz *et al.* (2012), whereby they noticed some COD removal occurred via physical means where particulate organic substrates were retained in the sludge bed. The growth and survival of microorganisms and anaerobic degradation of organic matter was found significantly affected by temperature. At decreasing temperatures, enzymatic and chemical reactions as well as the microbial growth were slowed down and the growth may stop at a very low temperature (Leitão *et al.* 2006).

The change in temperature also affects the BOD concentration profile. Throughout the reactor, it can be seen in Figure 6 that the BOD concentrations (in order from influent, Compartment 1 to 5 and effluent) were 1,026, 1,020, 97, 88, 78, 78 and 63 g/L for low mesophilic; 1,095, 513, 85, 54, 42, 36 and 28 mg/L for mesophilic; 1,110, 267, 252, 192, 187, 189 and 187 mg/L for thermophilic. The BOD removal and concentration profiles followed the same trend of COD. The BOD concentration in Compartment 1 was very high during the mesophilic conditions but it was distributed over the reactor system in thermophilic conditions. Nevertheless, the removal efficiency of BOD slightly increased from 93 to 97% when the temperature was increased from 29 to 37 °C, while it decreased from 97 to 83% with the increase in temperature from 37 to 55 °C.

Change in temperature surely affected the organic matter removal, so this led to a change in BOD removal. As the temperature increased, the removal of organic matter also increased until a certain limit. But, temperature variations within operation (whether increase or decrease) may harm the system. Decrease in removal efficiency of BOD might be due to the inhibition of microorganisms at high temperature. It needs nearly constant temperature for start-up as well as for continuous performance. Mustapha *et al.* (2003) have reported the BOD removal efficiency during palm oil mill effluent treatment using a thermophilic up-flow anaerobic filter. They revealed that the BOD removal decreased from 97 to 65% when the temperature was increased from 37 to 55 °C.

A decrease in BOD removal efficiency was also found associated with a decrease in the average VSS concentration in the reactor at thermophilic temperatures. Barr *et al.* (1996) have reported the treatment of kraft pulping effluent using an activated sludge bioreactor, and they found out that a decrease in removal efficiency of BOD (95 to 87%) was noticed at 44 °C was due to a significant decrease in concentration of VSS (from 1,700 to 1,000 mg/L). They also observed a higher dissolution of organic constituents at a high temperature of 50 °C. However, the reduction in BOD removal and VSS concentration might be due to the protein denaturation at thermophilic temperatures (Li *et al.* 2015).

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The pH, VFA and alkalinity levels in the reactor would reflect the degree of acidification in an anaerobic system. The system's response to temperature change was monitored from the point of pH profiles variations. Throughout the reactor, Figure 7 shows the pH profiles (in order from influent, Compartments 1 to 5 and effluent) were 6.8, 6.2, 6.21, 6.22, 6.23, 6.28 and 6.31 for low mesophilic; 6.86, 6.41, 6.42, 6.45, 6.47, 6.47 and 6.5 for mesophilic; 6.75, 6.28, 6.29, 6.37, 6.39, 6.4 and 6.41 for thermophilic. The metabolic processes' separation in the MAIB was reflected by low pH level in the front compartments, where most organic acids accumulated due to the activity of fermentative bacteria, and a slightly neutral pH level in the rear compartments where conversion of the acids into biogas led to increased alkalinity.

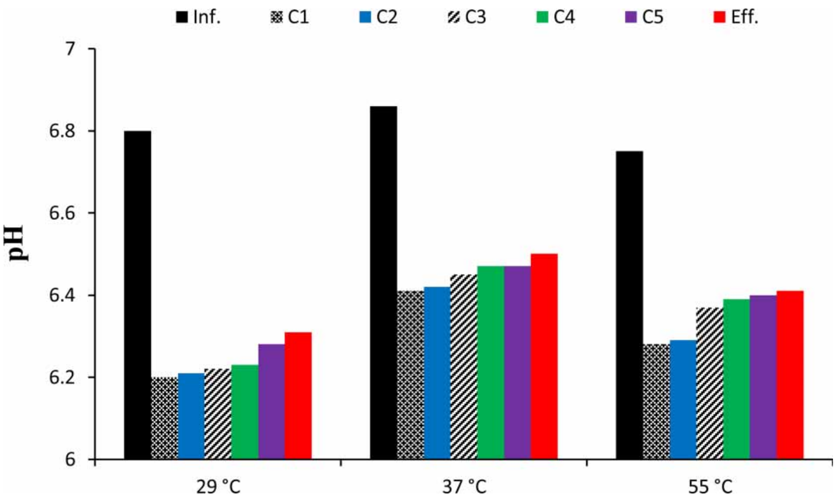


**Figure 7** | Characterization of pH according to MAIB reactor compartments during steady state at different temperatures.

In this context, Ziganshin *et al.* (2019) also proved the spatial separation of metabolic stages in the anaerobic baffled reactor (ABR), and the pH level increased throughout the system. In addition, the pH variation after the increase of temperature was not too significant due to higher microbial activities, which is a result of the balance between fermentation bacteria and methanogens. An average of 5 compartments' pH levels in mesophilic conditions (6.53) was slightly higher than those of thermophilic (6.44) and low mesophilic (6.23) conditions. The reason could be high activity of acidogenic bacteria in the low mesophilic and thermophilic conditions (Huang *et al.* 2015).

As shown in Figure 8, the VFA concentration profiles throughout the reactor (in order from influent, Compartments 1 to 5 and effluent) were 265, 72, 47, 23, 19, 16 and 17 mg/L for low mesophilic; 250, 100, 65, 42, 36, 33 and 28 mg/L for mesophilic; 245, 236, 198, 158, 149, 147 and 139 mg/L for thermophilic. The results indicated that the VFA value of thermophilic conditions is higher compared to low mesophilic and mesophilic conditions.

Amino acids and monosaccharides are converted into VFAs by the activity of acidogenic bacteria during anaerobic fermentation. Higher operating temperature has improved the hydrolysis of RPMW, resulting in augmented soluble carbohydrate and protein concentrations. Due to higher activities of fermentative bacteria at 55 °C, relatively sufficient soluble substrates were efficiently converted into VFAs, compared to mesophilic temperatures (Cho *et al.* 2015). A similar trend was also reported by Suhartini *et al.* (2014) during the anaerobic digestion of sugar beet pulp at mesophilic and thermophilic conditions. Their results showed that the VFA concentration in thermophilic conditions was higher (503 mg/L) compared with mesophilic conditions (23 mg/L). It was also reported that the maximum VFA generation was obtained above 50 °C (Elefsiniotis *et al.* 2005).

With regard to the alkalinity, Figure 9 shows the profiles of alkalinity throughout the system (in order from influent, Compartments 1 to 5 and effluent) were 220, 360, 375, 380, 400, 405 and

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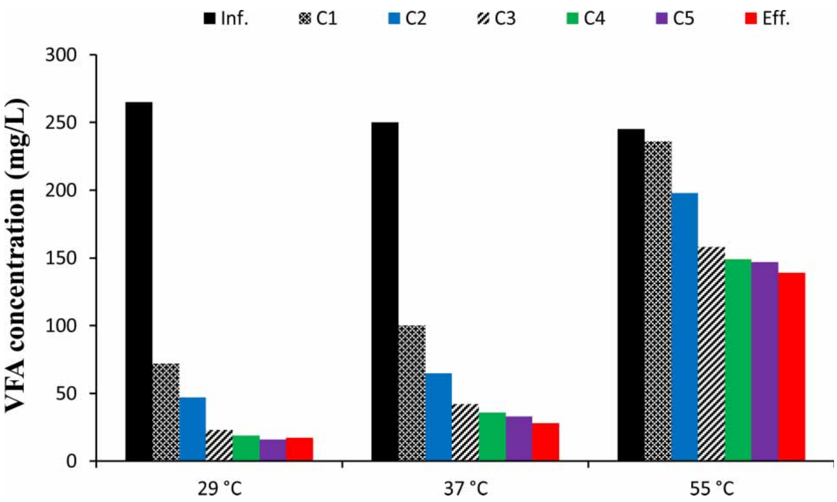


**Figure 8** | Characterization of VFA according to MAIB reactor compartments during steady state at different temperatures.

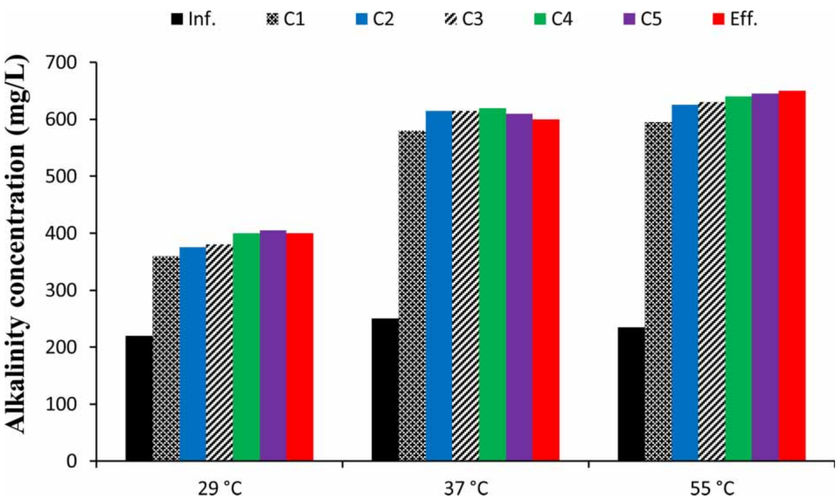


**Figure 9** | Characterization of alkalinity according to MAIB reactor compartments during steady state at different temperatures.

400 mg/L for low mesophilic conditions; 250, 580, 615, 615, 620, 610 and 600 mg/L for mesophilic conditions; 235, 595, 625, 630, 640, 645 and 650 mg/L for thermophilic conditions. It was noticed that the alkalinity concentration increased as the temperature increased. This result was analogous with that reported by Bouallagui *et al.* (2009), whereby the alkalinity concentration increased as operating temperature increased. The alkalinity generated during treatment is primarily produced by the mineralization of protein into ammonia, which latter combines with the carbonic acid in solution to form an ammonium bicarbonate buffer (Massé & Masse 2001). A higher protein mineralization at higher temperature may account for the difference in alkalinity in MAIB compartments and effluent. Furthermore, the reaction of carbon dioxide with ammonia and water may form ammonium bicarbonate, resulting in the formation of bicarbonate alkalinity in the reactor (Zhao & Viraraghavan 2004). However, alkalinity reduction was not encountered in this study, indicating sufficient buffering capability of the reactor.

## CONCLUSION

Operational temperature is a crucial factor influencing the performance of the MAIB reactor treating RPMW. A high total COD removal was attributed to biodegradable COD removal by biodegradation

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process of about 49% and non-biodegradable COD removal by settlement of organic matter of about 51%. Mesophilic temperatures led to high COD removal due to the combination of physical accumulation of organics and anaerobic digestion, where Compartment 1 and Compartment 2 had the highest sludge accumulation in the system. COD removal was decreased due to the solubility of organic compounds at thermophilic temperatures. Solubility increased the available COD for anaerobic digestion, which required a longer reaction period and higher microbial community activity. This was indicated by pH level, which was stable during mesophilic conditions and dropped but gradually increased in thermophilic conditions. In addition, thermophilic conditions yielded higher methane compared to mesophilic temperatures. Hence, treating the RPMW at thermophilic temperatures is more effective but requires careful operational control, while RPMW treatment at mesophilic temperatures is more stable but results in accumulation of solids inside the reactor.

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## CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

## DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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