Volatile fatty acid (VFA) removal of anaerobically digested molasses wastewater (MWW) in aerobic sequencing batch reactor (SBR) and up-flow aerobic column reactor (UACR) under various hydraulic retention time (HRT)

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Abstract. This paper analyses the removal of volatile fatty acid (VFA) of anaerobically digested molasses wastewater (MWW) in three different phases, which are acclimatization phase, aerobic sequencing batch reactor (SBR) phase and up-flow aerobic column reactor (UACR) phase. The UACR was modified from SBR by recycling the effluent to the influent tank for circulation purpose. The effect of hydraulic retention time (HRT) was determined during the operation of UACR. The influent tank was filled with 1.0, 2.0, 3.0, 4.0 and 5.0 L of anaerobically digested MWW which corresponds to HRT 14, 7, 4.7, 3.5, and 2.8 days. The operation of SBR achieved 85.4 ± 1.8 % of VFA removal at HRT 14 days. When modified to UACR, the VFA removal efficiency reached 85.5 ± 2.3 % at HRT 14 days. The VFA removal changed from 85.5 ± 2.3, 81.0 ± 0.4, 81.3 ± 2.2, 84.6 ± 5.1 to 87.4 ± 0.7 % in the UACR when the HRT decreased from 14 to 2.8 days. The UACR achieved optimum VFA removal at HRT 2.8 days and had greater performance when compared to SBR since it required shorter aeration time to obtain similar result.

1. Introduction
Molasses distillery industries are known as one of the key contributors to the economy. However, the production from the molasses distillery industries contributes to the environmental pollution in the water bodies [1]. The high strength molasses wastewater (MWW) produced has high chemical oxygen demand (COD) and biochemical oxygen demand (BOD) which are not suitable to be discharged without proper treatment. Apart from low pH, the MWW can be characterized to have dark brown colour, extensive odour, high concentration of difficult biodegradable, recalcitrant organic, and inorganic compounds [2]. In recent years, there has been an increasing interest in sequencing batch reactor (SBR) in conventional wastewater treatment. It is known as the activated sludge process that can be applied in high strength wastewater treatment [3]. SBR involves the functions of equalization, treatment, and sedimentation, which operates in time rather than in space. The processes in the SBR enhance the stabilization of waste and also the separation of suspended solid from the wastewater. Therefore, SBR is known as a promising
biological treatment method due to the process flexibility with the single tank design that fulfills different treatment requirements [4].

This research was focusing on the VFA removal in the acclimatization phase, operation of SBR, and operation of UACR. The SBR was modified to UACR to achieve better circulation in the reactor. The effect of HRT on VFA removal was investigated during the operation of UACR. The comparison of the performance on VFA removal was determined between SBR and UACR at HRT 14 days. There is limited information regarding the VFA removal in aerobic treatment compared to anaerobic digestion system. This paper can reveal the effectiveness of aerobic SBR and UACR in removing the remaining VFA concentration of MWW after undergoing the anaerobic digestion.

2. Methodology

2.1 Seeding sludge and wastewater

The seeded sludge was collected from the anaerobic pond in Malpom Industries Berhad, Penang, while the MWW was taken from the discharge pond in Fermpro Sdn. Bhd, Perlis. The wastewater samples were preserved in the cold fridge at a temperature of $4 \pm 1^\circ C$ which is above the freezing point to prevent the wastewater from biodegradation due to microbial action.

2.2 Reactor set up

Figure 1 and figure 2 illustrated the schematic diagram of aerobic SBR and UACR. The cylindrical reactor has 70 cm height and 15 cm internal diameter. The total volume of the reactor is 14 L and the working capacity is 12 L. Atinan HP-4000 aquarium air pumps was used for aeration supply. The aeration bar was placed at the bottom of the reactor for the aerobic condition. A peristaltic pump was used to feed the influent into the SBR, while the air flow rate was fixed by using air-flow meter. Wave maker was used for the circulation and mixing of the MWW during the react phase. The processes involved in the reactor was controlled by timer. UACR was modified from SBR by discharging the effluent to the influent tank for circulation of wastewater continuously.

2.3 Operation of SBR

The SBR was operated for 30 days at HRT 14 days. The SBR operation involves four stages of fill (0.5 h), react (21 h), settle (3 h), and draw phase (0.5 h), which one cycle in 24 hours. Air was supplied during the react stage in air flow rate of 100 mL/min for 21 hours and operated under room temperature ($28 \pm 2^\circ C$). A volume of 1 L feed solution was introduced into reactor during the fill stage, and the same amount of effluent was drawn during the decant stage after 3 hours of settling.

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**Figure 1.** Schematic diagram of aerobic sequencing batch reactor (SBR).
2.4 Operation of UACR
UACR was operated under various HRT to evaluate the performance in treating MWW. The influent tank was filled with 1.0, 2.0, 3.0, 4.0 and 5.0 L of MWW which corresponds to HRT 14, 7, 4.7, 3.5, and 2.8 days in 50 days. The UACR was operated for 24 hours which involved 4 running cycles of feed (0.5 h), react (18 h), settle (6 h) and draw (0.5 h). The effluent from the top valve was returned into the influent tank as the recycled influent. Air was supplied at the react stage at constant flow rate of 100 mL/min. The cycle of feed and react was carried out simultaneously in 18 hours. Lastly, the cycle was allowed for 6 hours of settle and the effluent was collected in the storage tank for the analysis.

2.5 Volatile fatty acid (VFA)
Measurement of volatile acidity as acetic acid content was determined according to Standard Method 5560 C distillation method. A recovery factor of about 70 % was determined using equation 1. A volume of 100 mL well mixed sample was placed in a 500 mL distillation flask together with 100 mL of distilled water and 5 mL of concentrated sulphuric acid (H\textsubscript{2}SO\textsubscript{4}). The sample was then mixed thoroughly and gently before connecting it to the condenser. The initial 15 mL of the distillate was collected in a 50 mL beaker was discarded and exactly 150 mL of distillate were collected in a 250 mL beaker. Phenolphthalein indicator was added to the collected distillate and the sample was titrated with 0.1 N sodium hydroxide (NaOH) until the colour changes to pale lavender or when reaching pH 8.3. Volatile acidity as acetic acid of the sample was calculated by equation 2.

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\text{Recovery factor, } f = \frac{U}{V} \quad (1)
\]

Where U is volatile acid concentration recovered in distillate (mg/L) and V is the volatile acid concentration in standard solution used (mg/L).

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\text{Volatile acidity, } \left( \frac{\text{mg of acetic acid}}{L} \right) = \frac{W \times Y \times 60000}{f \times \text{volume of sample, mL}} \quad (2)
\]

Where W is the volume of titrant to reach end point (mL), Y is the equivalents NaOH per litre titrant and f is the recovery factor.
3. Result and discussion

3.1 Acclimatization phase
The volatile fatty acid is a short-chain fatty acid generated during the biohydrogen process through anaerobic fermentation [5]. In addition, VFAs are vital intermediary compounds in the metabolic pathway of methane fermentation. The VFA can be used external carbon sources for biological nutrient removal such as nitrogen and phosphorus in the wastewater treatment plant [6]. The VFA concentration of influent was $1101 \pm 570$ mg/L, while the seeded sludge used for the acclimatization phase was 8106 mg/L. According to figure 3, the initial concentration of VFA in the SBR is 3383 mg/L, which was higher than the influent. The concentration of VFA rose gradually to 5223 mg/L in day 3. The accumulation of the VFA can cause depletion of buffering capacity and depression of pH. The concentration of VFA decreased gradually to 800 mg/L at day 14. The trend between VFA and COD was similar because when the effluent COD concentration increased, the VFA concentration also increased [7].

![Figure 3. VFA concentration in the aerobic SBR during acclimatization phase.](image)

3.2 Operation of Sequencing Batch Reactor (SBR)
Figure 4 shows the VFA concentration and VFA removal efficiency during the operation of aerobic SBR at HRT 14 days.

![Figure 4. VFA concentration and removal efficiency during the operation of aerobic SBR at HRT 14 days.](image)
The range of VFA concentration in the effluent was between 142 and 205 mg/L with the average concentration of 160 ± 20 mg/L. The VFA removal efficiency was 85.4 ± 1.8 % during the operation of SBR. The removal of VFA concentration was high because the VFA can be used as the carbon source for biological nutrient removal in the SBR [8]. The VFA concentration in the treated effluent was 206 mg/L at day 1. The VFA concentration was reduced to 171 mg/L at day 4 and remained stable from day 4 to day 10. The reduction of VFA concentration resulted continuously from day 10 to day 20, which declined to 144 mg/L. The concentration of VFA further declined slightly to 143 mg/L in 30 days of operation by using SBR. The decrease of the VFA concentration in the aerobic SBR effluent throughout the operation may be due to the production of alkalinity to bicarbonate [9]. This finding was supported by Bernet et al. (2000) [10] that aerobic sequencing batch reactors enable efficient removal of the volatile fatty acids concentration with 100% removal efficiency. VFA can be consumed as the carbon source during the denitrification process in the reactor [11].

3.3 Operation of up-flow aerobic column reactor (UACR)

Figure 5 showed the variation of VFA concentration in various HRT. The VFA concentration was evaluated at HRT 14, 7, 4.7, 3.5, and 2.8 days respectively. The overall VFA concentration for the treated effluent during the operation of UACR was in the range of 131 to 246 mg/L. At HRT 14 days, the concentration of the VFA in the treated effluent was ranged between 143 to 211 mg/L with an average removal of 85.5 ± 2.3 %. The removed VFA was efficiently converted into PHA because it is easily biodegradable organic compounds [12]. During the treatment, VFAs was taken up by the PHA-storing bacteria for PHA accumulation and growth [13]. The concentration of the VFA in the effluent was 209 ± 4 mg/L on operation day 22 to day 28 at HRT 7 days. The reduction of the VFA concentration was 81.0 ± 0.4 % that indicated a slightly decrease of degradation rate in VFA concentration. When the HRT decreased from 7 to 4.7 days, the VFA removal efficiency increased slightly to 81.3 ± 2.2 % with the average concentration of 206 ± 24 mg/L. The operation with prolonged HRT enabled the accumulation of VFA in steady conditions [14].

The VFA concentration in the treated effluent was 169 ± 57 mg/L with the VFA reduction of 84.6 ± 5.1 % at HRT 3.5 days. The concentration of the VFA at HRT 2.8 days achieved better removal, which was 87.4 ± 0.7 %. When compared VFA to the others sugar-based substrates, VFA has higher lipid conversion efficiencies because it has shorter metabolic pathways [15]. Thus, the reduction of the VFA reached 85.5 ± 2.3 % in this research, which due to the characteristic of easy biodegradable in the treatment. Thus, the decreasing of HRT might lead to higher VFA reduction because the loading rate of VFA concentration increased. According to Lee et al. (2014) [16], the distribution of VFA was usually significantly affected by the effect of pH and HRT. However, the removal of VFA concentration in anaerobically digested MWW in UACR was not significantly affected by the effect of HRT since the overall VFA removal was 84.5 ± 3.5 % which was considered stable in all HRT.
3.4 Comparison on the effectiveness of the SBR and UACR in VFA removal under aerobic conditions at HRT 14 days

From the figure 6, the effectiveness of SBR and UACR on the VFA concentration and removal efficiencies was determined at HRT 14 days. The aerobic SBR yielded 85.4% of VFA reduction and 161 mg/L of VFA concentration with the aeration time of 21 hours while UACR with aeration time of 18 hours achieve 85.45% of VFA reduction and 160 mg/L of VFA concentration. The VFA was removed by converting to the PHA, as the VFA can be used as raw substance [17]. The VFA reduction of both reactors achieved similar result when SBR was modified to UACR. Therefore, it can be concluded that the performance of UACR was better than SBR because UACR required shorter aeration time to achieve similar result. According to Llamas et al. (2020) [18], VFA from anaerobic fermentation was consumed in the yeasts growth as carbon source, which was proved by the VFAs profile in the study. VFA can be used as readily biodegradable carbon sources for biological nutrients removal, which is important for the growth of microorganisms in the reactor [19]. Therefore, VFA can be used as an important carbon source in the treatment process and the feasibility study of both bioreactors was required to determine the optimization of operational parameters in the MWW treatment.

Figure 5. VFA concentration and VFA removal efficiency during the operation of UACR.

Figure 6. VFA concentration and removal efficiency during the operation of SBR and UACR at HRT 14 days.
4. Conclusion
This research was focusing on the VFA concentration in the acclimatization phase, operation of SBR, and operation of UACR. VFA concentration was reduced gradually during the acclimatization phase. During the operation of SBR, the reduction of VFA concentration was 85.4 ± 1.8 % at HRT 14 days. The effect of HRT on VFA removal was investigated during the operation of UACR. The overall VFA concentration during the operation of UACR was in the range of 131 to 246 mg/L. UACR achieved the maximum VFA reduction at HRT 2.8 days, which was 87.4 ± 0.7 %. When comparing the performance between SBR and UACR, the UACR achieved greater performance since it required shorter aeration time to obtain similar result. The optimization of the operational condition like aeration time and settling time can be determined to improve the VFA removal under the SBR and UACR operation.

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