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Impacts of different operational temperatures and organic loads in anaerobic co-digestion of food waste and sewage sludge on the fate of SARS-CoV-2

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ABSTRACT

The impacts of different operational temperatures, and organic load (OL) on the fate of SARS-CoV-2 during the anaerobic co-digestion of food waste (FW) and sewage sludge (SS) was evaluated. The lab-scaled batch reactors (i.e. R1-R7) were performed under psychrophilic, mesophilic, and thermophilic conditions and the OL of systems was 1.5, 3.5, 6 gVS/L. The performance parameters showed that at higher OL the stability of systems failed and low biogas was produced. In contrast, increasing of operational temperature of systems induced more biogas generation due to the increment of metabolic activity of bacteria. Therefore, R1-R7 achieved biogas yield of 202.5, 249, 187, 260, 246, 163, and 300 mL/gVS respectively. Both SARS-CoV-2 genes i.e. ORF1ab, and N genes were detected in the effluent of psychrophilic reactors i.e. R1, and R2, with a total concentration of $46 \times 10^3$ and $11 \times 10^3$ copies/L respectively. In R3, no viral genes were observed, when the VFAs was accumulated up to 2000 mg/L and caused a pH drop to 5.6. At the mesophilic condition, the viral concentration was significantly declined, and no viral genes were observed at an OL of 3.5 gVS/L. Furthermore, the synergistic effect of temperature and accumulation of intermediate metabolites provided a sever condition for SARS-CoV-2 survival at an operational temperature and OL of 50 °C, and 1.5 gVS/L respectively.

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1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic has become the current ongoing global issue (Saba and Elsheikh, 2020; Collivignarelli et al., 2020) involved 216 countries with over 33 million of the infected cases and more than one million deaths worldwide, as of September 29, 2020 (WHO, 2020). According to the clinical investigation, the symptoms in SARS-CoV-2 patients mainly include difficulty in breathing followed by severe fever, cough, and diarrhea (Gao et al., 2020; Kumar et al., 2020a). From the recent investigation, it was reported that the RNA of SARS-CoV-2 was detected in the patient’s feces (Cheung et al., 2020). A SARS-CoV-2 patient, on average, can effluent about 600,000 viral genomes per mL through fecal material (Wu et al., 2020). These huge amounts of viral particles are discharged in sewage and consequently end up in wastewater treatment plants (WWTP) (Venugopal et al., 2020). To date, the presence of SARS-CoV-2 RNA in wastewater in Italy (Rosa et al., 2020), Netherlands (Medema et al., 2020; Lodder and de Roda Husman, 2020), USA (Wu et al., 2020), France (Wurtzer et al., 2020), India (Kumar et al., 2020b), and Australia (Ahmed et al., 2020) was verified.

Furthermore, the study on the presence of SARS-CoV-2 RNA in WWTP in Turkey has shown that about 1.17–4.02 × 10^4 viral genomes per liter have existed in primary and secondary sludge (Kocamemi et al., 2020). Although the residence time of SARS-CoV-2 in WWTP has not been elaborately studied, the recent evidence has shown that SARS-CoV-2 can survive in WWTPs for a long period of time (Kumar et al., 2020b). It was found that the presence of bacteriophages in sewage sludge can help the virus to survive through providing a cell culture for virus propagation (Venugopal et al., 2020). The survival parameters of SARS-CoV-2 in the aqueous environment include temperature, pH, light exposure, total dissolved solids, nitrate concentration, organic matter, turbidity, presence of antagonist organisms, and hardness (John and Rose, 2005; Naddeo and Liu, 2020). Moreover, it was purported that SARS-CoV-2 can be transmitted through airborne transmission in WWTPs (Zhang et al., 2020). Also, the possibility of the pollution of the drainage system by SARS-CoV-2 contaminated wastewater was verified (China Citywater, 2020). Therefore the WWTPs are the
destination of SARS-CoV-2, and it is assumed that the other part of WWTP, can be contaminated.

To date, no detailed study was carried out on the fate of SARS-CoV-2 in the anaerobic digestion process. It is known that anaerobic digestion can reduce the pathogens and viruses due to the various biochemical reactions that are taken place during the anaerobic digestion process. Therefore, for the first time, this study investigates the impact of different temperature conditions and organic loads (OL) in anaerobic co-digestion (AcoD) of food waste (FW) and sewage sludge (SS) on SARS-CoV-2 via real-time PCR technology (RT-PCR).

2. Material and methods

2.1. Substrate, viral urine, and inoculum

The characteristics of FW and SS are similar to previous studies (Bardi and Rad, 2019; Bardi and Aminirad, 2020). FW was collected from the dormitory restaurant of Babol Noshirvani University of Technology, Iran. The collected FW was shredded into small pieces (<2 mm), milled to homogenize, and kept at 4 °C prior to using. The SS was taken from WWTP of Babol, Iran. The SS was filtered to remove impurities and coarse solids and kept at 4 °C prior to use. The characteristics of FW and SS are summarized in Table 1. The inoculum of AcoD was taken from an Up-flow anaerobic sludge blanket (UASB) reactor treating dairy sludge with characteristics of soluble chemical oxygen demand (SCOD) of 298 mg/L, volatile solids of 3150 mg/L, and pH of 7.24. The viral urine was collected by the Foley Catheter bag of SARS-CoV-2 patient from Babol Hospital, Iran. The fresh urine with viral particles of 52 ± 4 × 10^6 copies/L was applied in the AcoD systems after collection and sets of sample processing, RNA extraction, and RT-PCR analysis.

2.2. Anaerobic co-digestion assay

In this study, the impacts of different temperatures condition and organic loads on SARS-CoV-2 was evaluated by bio-methane potential (BMP) tests. Seven reactors namely R1-R7 were designated with psychrophilic (i.e. 20 ± 2 °C), mesophilic (35 ± 2 °C) condition, at different OLs of 1.5 gVS/L, 3.5 gVS/L, and 6 gVS/L, respectively as well as thermophilic (50 ± 3 °C) temperature with an OL of 1.5 gVS/L. Since the aim of this study is to evaluate the fate of SARS-CoV-2 at the end of AcoD process, the selection of OLs is based on the recent studies in which this range of OL was considered for the operation of conventional AcoDs (Bardi and Rad, 2019; Bardi and Aminirad, 2020; Gnouci et al., 2020; Musa et al., 2018; Logan et al., 2019). Seven control samples (i.e. C1-C7) were performed at different temperatures conditions and organic loads without the addition of inoculum to evaluating the impacts of SS, FW, and temperature on SARS-CoV-2. Table 2 shows a summary of reactor operating conditions in BMP tests. The BMP tests were carried out in the 300 mL glass bottles reactor with a working volume of 250 mL each. The reactors were connected to the water chamber for measuring the biogas volume via water displacement method, and a magnetic stirrer with heater (RT10-Magnetic Stirrers, IKA) was used to provide mixing of 50 rpm. The schematic picture of anaerobic digester is shown in the supplementary file. The concentration of SS was 9500 mgVS/L in each reactor, while the different OLs of FW were applied to the digesters (e.g. the ratio of SS to FW was 6.3, 2.7, and 1.6 respectively based on VS). The TS of R1-R7 was 16.13 g/L, 18.42 g/L, 21.35 g/L, 16.15 g/L, 18.40 g/L, 21.28 g/L, and 16.14 g/L, respectively. Also, 100 mL of viral urine (e.g. 20 ± 3.5 × 10^6 copies/L) was injected in each reactor. The ratio of inoculum to the substrate was 0.1 (V/V) in all reactors and the nitrogen gas was used to purge the reactors. All experiments were performed and analyzed in triplicate.

2.3. Analytical methods

The parameters i.e. SCOD, COD, alkalinity, TS, and VSS were analyzed according to Federation, 2005. The metal content of substrates was determined using the Microwave Plasma Atomic Emission Spectrometer (MP-AES, Agilent 4100, America). Chinese standard methods (Chinese National Standard, 2010a,b) were used to measuring Protein, carbohydrates, lipids, cellulose, and calcium of substrates. 2 mL of sample was enough to analyze the VFAs and the pH of the solution. 0.1 mL of the extracted sample was injected into GC (Agilent 6890 N, USA) equipped with a flame ionization detector, DB-wax capillary column (Agilent Technologies, USA), and helium gas as a carrier (flow rate of 4 mL min − 1) to determining the characteristics of VFAs (Bardi and Rad. 2019). The pH of samples was measured using a pH meter (PCE-PHD 1). An element analyzer (Vario EL III, Elementar, Germany) was used to determine the ratio of C, and N in the organic substrates. The content of CO₂ and CH₄ in biogas was determined by gas chromatograph (GC) (Agilent Technologies 6890 N, USA). All samples were analyzed in triplicate.

2.4. Sample processing and RNA extraction

In this study, the polyethylene glycol (PEG) precipitation of centrifuged supernatant was used as an RNA extraction method (Kumar et al., 2020b). First, 50 mL of each sample was centrifuged (Model: NuWind NU-C300R) at 4500 g for 30 min. Then the supernatant was filtered using 0.22 μm filters (Cellulose Esters Membrane, Sterile). Each filtrate samples (25 mL) was concentrated through the addition of PEG 9000 (80 g/L) and NaCl (17.5 g/L), and then incubated (Model: KS 4000 I control Incubator shaker, IKA) at 17 °C and 100 rpm. The mixture was centrifuged (Model: NuWind NU-C300R) at 13,000 g for 90 min. on the following day. After centrifugation, the pellet was added into 300 μL RNase-free water, while the supernatant was discarded. RNA was directly extracted using a kit (NucleoSpin® RNA Virus, Macherey-Nagel GmbH & Co. KG, Germany). For evaluating the efficiency of RNA isolation and RT-PCR inhibition, 200 μL of concentrated samples were mixed with 10 μL MS2 phage (i.e. for process control), 20 μL Proteinase K solution and 600 μL of RAV1 buffer as a carrier RNA according to (Haramoto et al., 2020). Further steps were done in accordance with the manufacturer’s protocols (Macherey-Nagel GmbH & Co.)
Table 2
Summary of reactor operating conditions in BMP tests.

| Reactors | Temperature conditions | Organic loads (gVSL/L) | Controls |
|----------|------------------------|------------------------|----------|
|          | 20 °C                  | 35 °C                  | 55 °C     |          |
| R1       | √                      |                        | 1.5 ± 0.13 | C1       |
| R2       | √                      |                        | 3.5 ± 0.2 | C2       |
| R3       | √                      |                        | 6 ± 0.25  | C3       |
| R4       | √                      |                        | 1.5 ± 0.16 | C4       |
| R5       | √                      |                        | 3.5 ± 0.23 | C5       |
| R6       | √                      |                        | 6 ± 0.3   | C6       |
| R7       | √                      |                        | 1.5 ± 0.1 | C7       |

KG). Qubit 4 Fluorometer (Invitrogen) was used to determine RNA concentrations.

2.5. RT-PCR analysis

RT-PCR was carried out to analysis of ORF1ab, and N gene of SARS-CoV-2 and MS2 using TagPath™ Covid-19 RT-PCR Kit (Applied Biosystems). The detection of SARS-CoV-2 by RT-PCR was investigated using the ORF1ab and N protein gene assay (Rimoldi et al., 2020). A 7 μL of extracted RNA of each samples was added in a 25 μL reaction mixture prior to amplification stage. 5 μL, and 2 μL of TagPath™ were used in negative and positive control respectively. In this study, Nuclease free water was considered as a batch control. Further procedures were done according with the manufacturer’s protocols. The RT-PCR cycling parameters consisted of 25 °C for 2 min incubation; 53 °C for 10 min for RT; 95 °C for 2 min; and 40 cycles of 95 °C for 3 s; and 60 °C for 30 s. The RT-PCR assays were performed using a Biosystems™7500 Fast Real Time PCR system (Applied Biosystem). For each RT-PCR test, three positive and negative controls were performed.

3. Results and discussion

3.1. Performance of AcoD at psychrophilic condition

The performance of AcoD was evaluated at a psychrophilic condition (20 °C) with different OLS. From Fig. 1A, the systems started to generate biogas within 48–52 hours of starting the experiment in R1–R3. It can be seen that as the OL of the system increased the biogas was sharply generated with a lower lag phase (Fig. 1A). This increment and quick biogas generation are due to the increase of metabolic activity of microorganisms involved in the AcoD process corresponding to the increase of the OL (Bardi and Aminirad, 2020; Castro-Molano et al., 2018). However, R1 and R2 produced biogas yield of 202.5, and 249 mL/gVS respectively, while, R3 with an OL of 6 gVS/L produced biogas production yield of 187 mL/gVS, despite sharp gas generation at an early stage (Fig. 1B). As can be seen from Fig. 1C, the stability of R3 in the biogas production failed due to severe pH decline at around 70 h of the experiment. On the other hand, R1 and R2 had a stable condition due to mild pH changes (Fig. 1C). It was reported that the operation of anaerobic digestion under low OL prevents the accumulation of inhibitory substances, representing stable performance and higher biogas production efficiency (Bardi and Rad, 2019; Bardi and Aminirad, 2020; Carboni et al., 2020). Furthermore, the methane and carbon dioxide content in biogas of R1–R3 was observed to be 67 ± 4%, 31 ± 2% and 66 ± 5%, 33 ± 1.5 % and, 61 ± 3%, 37 ± 2% respectively. The lower content of methane in the produced biogas is related to severe pH to decline at higher OL, where the excessive acidification of digesters leading to more CO2 production over CH4 (Feng et al., 2018).

Considering the VFAs generation and conversion, R1 with an OL of 1.5 gVS/L, represented a favorable condition for VFAs conversion. The acetic acid (HAc) was sharply produced until around 70 h, peaked at 681 mg/L. Since then, however, HAc concentration has reduced to 320.3 mg/L as a final concentration (Fig. 2R1). During this period (i.e., 70–150 hours of the experiment), the pH of R1 increased because of the reversible VFAs conversion (Fig. 1C). In R2, a higher OL (i.e. 3.5 gVS/L) induced more VFAs accumulation in forms of HAc and propionic acid (HPr) (Fig. 2R2). However, the concentration of HAc (828 mg/L), and HPr (369 mg/l) in R2 was within the permissible range for the activity of methanogenic bacteria (González et al., 2018). On the other hand, the increase of OL to 6 gVS/L, exhibited severe HAc and HPr accumulation up to 1536, and 766 mg/L respectively at around 125–150 hours of the experiment (Fig. 2R3). The VFA to alkalinity ratio (VFA/ALK) is another performance parameter of AcoD implies that the ratio under 0.4 guarantees the stability of the system against excessive acid-forming, while the ratio within 0.4–0.8 induces minor instability, and the ratio behind 0.8 can cause severe inhibition and system failure (Rasapoor et al., 2020; Bardi and Aminirad, 2020).

The ratio for R1, and R2 was relatively under 0.4 throughout the AcoD process, representing no inhibition caused by VFAs accumulation, whereas in R3, the ratio was above 0.4 after 70 h of the stopping of the experiment (Fig. 1D). Therefore, the pH of the system (R3) declined to 5.55 (Fig. 1C) and provided the thermodynamically unfavorable condition for VFAs conversion until the end of the experiment (Capson-Tojo et al., 2017).

3.2. Performance of AcoD at mesophilic, and thermophilic conditions

The performance parameters of AcoD under mesophilic condition were relatively similar to the thermophilic state. In all reactors biogas was generated at around 13 h (except for R4), and its sharp generation lasted until around 20 h, but thereafter its generation declined, and no biogas was produced after 46 h (Fig. 3A). As a result, the biogas production yield of 260 mL/gVS, 246 mL/gVS, 163 mL/gVS, and 300 mL/gVS was recorded in the reactors R4–R7 respectively (Fig. 3B). Interestingly, a higher application of OLS, as well as temperature conditions, resulted in quick biogas generation compared with the psychrophilic state due to an increase in metabolic activity of microorganisms (Castro-Molano et al., 2018). Also, the lag phase of digesters (i.e. R4–R7) in biogas production was significantly lower than that of other AcoD investigations (Gaby et al., 2017; Wu et al., 2018; Xiao et al., 2018, 2019). This could have been due to the stimulatory impacts and readily characteristics of FW (Bardi and Rad, 2019), the stimulatory effects of efficient trace elements in SS (Table 1) on the wide range of enzymatic reactions (Bardi and Aminirad, 2020; Wang et al., 2020a) or the inoculum adaptability and strength. The biogas production yield in R7 (thermophilic condition) despite a low organic load (i.e. 1.5 gVS/L) was higher than that of other reactors. This phenomenon could have been due to higher efficiency in organic materials degradation, and an increase of intermediate metabolism during thermophilic AcoD (Liu et al., 2017). From Fig. 3C, as the OL was increased the pH of systems was declined, and AcoD interfaced with the unstable con-
Fig. 1. The performance parameters of R1-R3 at different organic loads and temperatures. A: biogas production, B: cumulative biogas yield, C: pH of system, D: VFA/ALK ratio.

Fig. 2. The concentration of VFAs in R1-R3.
dition for biogas generation. The VFA/ALK ratio for R4, and R7 was constantly below 0.4 during the AcoD process, while the ratio for R5 increased to around 0.5 after 38 h of the starting of the experiment. Therefore, the accumulation of VFAs during this period (i.e. 38–45 hours) caused R5 instable for biogas production. On the other hand, the quick VFAs generation in R6 represented the VFA/ALK ratio greater than 0.8 after around 25 h of starting the experiment, led to sever pH to drop, and resulted in a stop process globally at an OL of 6 gVS/L. To describe this phenomenon, on the one hand, the applied FW was rich in easily biodegradable materials and various micro/macro elements that stimulate the activity of organisms for its degradation, but on the other hand, since the metabolic activity of methanogenesis bacteria is significantly lower than that of aci-dogenesis bacteria, the VFAs generation rate is more quickly than its conversion (Fu et al., 2019; Wintsche et al., 2018), resulting in accumulation of VFAs, sever pH to drop, and system failure. With respect to this, the CO₂ content increased in the produced biogas from 33 ± 4%–42 ± 6% in the R4-R6 as a result of acidification. However the content of CO₂ and methane in the R7 was similar to the psychrophilic and mesophilic conditions due to the stable performance.

From Fig. 4, the pattern of VFAs production in mesophilic, and thermophilic conditions was relatively similar to the psychrophilic state. In R4, and R7, the HAc, and HPr were accumulated up to 1135.5 mg/L, 1736 mg/L, and 1562 mg/L, 182 mg/L respectively until around 25 h. Thereafter, however, both reactors favored the reversible VFAs conversion under stable conditions. Thus, in R4 and R7 the HAc, and HPr were declined to below 345 mg/L, 269 mg/L, and 541 mg/L, 241 mg/L respectively at the end of the AcoD process. Although the OL of R1, R4, and R7 was similar (i.e. 1.5 gVS/L), the higher VFAs accumulation at an early stage of the experiment was correlated with the higher temperature condition in which the higher and fast degradation of organic materials is facilitated (Adekunle and Okolie, 2015; Akbar et al., 2020). In R5 and R6, the figure for VFAs conversion was similar, where the HAc and HPr were accumulated up to 1523 mg/L, 789 mg/L, and 2459 mg/L, 1882 mg/L respectively. It was declared that the accumulation of HAc and HPr up to 2400 mg/L, and 900 mg/L can hinder the activity of methanogenesis bacteria and resulted in stop process globally (González et al., 2018). Therefore, the OL of 6 gVS/L in R6 caused excessive VFAs accumulation behind a permissible level and re-presented sever inhibition at an early stage of the experiment.

3.3. RT-PCR performance and results

The two genes i.e. N protein and ORF1ab genes of SARS-CoV-2 were examined from the effluent of different AcoDs and control samples. The molecular process inhibition during the RT-PCR reaction was verified using MS2 in each sample and negative control. None of AcOD and control samples showed a significant inhibition, as confirmed by the MS2 RT-PCR assay, where the amplification cycles (CT) value in all samples i.e. R1-R7 were 27.89, 27.66, 27.12, 27.69, 27.20, 27.16, and 27.05, respectively, and C1-C7 were 26.88, 26.52, 26.22, 26.98, 26.16, 26.33, 26.14 respectively.

According to Table 3, some samples were positive with the ORF1ab, and N genes, whereas the others were negative in both ORF1ab and N genes. The samples i.e. R1, R2, and R4 were positive with all genes, however, their maximum concentrations were varied. In R1, the smaller CT value implies the abundant of all SARS-CoV-2 genes in the effluent of AcoD with maximum concentrations of \(46 \times 10^3\) copies/L. Next to that, R4 with the maximum concentration of \(29 \times 10^3\) copies/L, and CT value of 35.49, and 35.19 for
ORF1ab, and N gene respectively, showed the abundant of viral genes in the effluent of AcoD. However, in R2 only \(1 \times 10^3\) copies/L with CT value of 36.21, and 36.68 for ORF1ab, and N genes respectively, was observed.

From Table 3, it can be noticed that higher OL in AcoD process induced a significant viral genes decline. R1 with an OL of 1.5 gVS/L had a virus concentration of \(46 \times 10^3\) copies/L, whereas, R2 with an OL of 3.5 gVS/L had a concentration of \(11 \times 10^3\) copies/L. Also, no viral particles were detected in R3 with an OL of 6 gVS/L. Since the anaerobic digestion consists of four stages namely hydrolysis, acidogenesis, acetogenesis, and methanogenesis, the formation and accumulation of the intermediate metabolites such as ammonia/ammonium, alcohols, formate, hydrogen, and VFAs are inevitable during anaerobic digestion (Bardi and Rad, 2019; Bardi and Aminirad, 2020; Capson-Tojo et al., 2017; Liang et al., 2020; Wang et al., 2020b). The formation and built-up of these metabolites can provide a sever condition for pathogens and virus survival (Decrey and Kohn, 2017; Seruga et al., 2020; Yang et al., 2019). The genome and core virion of viruses are protected with an envelope that plays a significant role in conveying the genome between host cells. Therefore, the denaturation of virus protein or disintegration of its lipid contributes to the disruption of the virus envelope and leads to the inactivation of the virus (Lin et al., 2020). In this regard, it was reported that the alcohol-based agent can cause protein denaturation in virus A/H1N1 by attacking to virus envelope (Grayson et al., 2009). From the recent study, it was denounced that some agents such as ethanol, propanol, and formaldehyde, povidone-iodine, and sodium hypochlorite, hydrogen peroxide with the concentration of 70–95 %, 0.7–1 %, and 0.21–0.5% respectively can significantly inactive coronavirus (Kampf et al., 2020). Thus, higher OL induced more accumulation of intermediate metabolites and pH to drop (Fig. 1), and this is consistent with viral genes decline in the effluent of reactors (Table 3). Therefore, no SARS-CoV-2 genes were observed in the effluent of

Table 3
Details of molecular detection of SARS-CoV-2 in the effluent of AcoD.

| Samples | Temperature (°C) | Organic load (gVS/L) | RT-PCR target region | Virus concentration (copies/L) |
|---------|----------------|-----------------------|----------------------|-------------------------------|
|         |                |                       | ORF1ab | N gene |                            |
| R1      | 20 ± 2         | 1.5 ± 0.13            | 34.33   | 34.66  | 46 ± 2 × 10⁴               |
| R2      | 20 ± 2         | 3.5 ± 0.2             | 36.21   | 36.68  | 11 ± 1.5 × 10⁴             |
| R3      | 20 ± 2         | 6 ± 0.25              | –       | –      | ND                           |
| R4      | 35 ± 2         | 1.5 ± 0.16            | 35.49   | 35.19  | 29 ± 4.5 × 10³             |
| R5      | 35 ± 2         | 3.5 ± 0.23            | –       | –      | ND                           |
| R6      | 35 ± 2         | 6 ± 0.3               | –       | –      | ND                           |
| R7      | 50 ± 3         | 1.5 ± 0.1             | –       | –      | ND                           |

ND no detectable.
R3, where the VFAs concentration and pH of the system were found to be above 2000 mg/L and below 5.4 respectively. Also, the viral genes were not detected in the effluent of the R5 where the VFAs concentration were below 2000 mg/L, although the pH of system was around 6.5. In fact, the acid-based aqueous solution can trigger the low pH-dependent change in the hemagglutinin and lead to aggregation and inactivation of hemagglutinin-glycoprotein spikes and virus (Sato et al., 1983). Furthermore, it was reported that the application of 10% malt vinegar (4–8% acetic acid) could reduce the viability of A/H1N1 strain of influenza (Greatorex et al., 2010). Similarly, it was demonstrated that acetic acid with a concentration of 5% can effectively inactivate A/H7N2 virus (Lombardi et al., 2008). Also, it was reported that the weaker acid form of acetic acid, i.e. peracetic acid can inactivate adenoivirus 8 within 5 min at a concentration of 0.2 % (Rutala et al., 2006). Thus, it can be concluded that the VFAs concentration up to 2000 mg/L followed by low pH conditions (i.e. 5.4) can disrupt the SARS-CoV-2 gene at an operational temperature of 20 °C with an OL of 6 gVS/L, whereas in mesophilic condition only 3.5 gVS/L is enough to eliminate the viral genes at the effluent.

Furthermore, from Table 3, it can be noticed that the operational condition (e.g. temperature, and OL) had a positive effect on the removal of SARS-CoV-2 genes at the effluent of AcoD. Although the OL in R4 and R7 was similar to R1 (i.e. 1.5 gVS/L), the concentration of SARS-CoV-2 was declined by 37% in R4, and no SARS-CoV-2 genes were detected in the effluent of R7. To prove our observation, it was already purported that prolonged incubation at 55 °C can effectively eradicate detectable infectivity (Greatorex et al., 2010). Also, it was reported that A/H7N2 influenza strain can be inactivated at 56 °C (Lancet, 2020). Furthermore, it can be concluded that the temperature state and concentration of the intermediate metabolites had a synergistic effect on SARS-CoV-2 removal (Lin et al., 2020). Thus, the VFAs accumulation above 2000 mg/L coupled with the operational temperature of 35 °C in R5 could eradicate the presence of SARS-CoV-2 genes in the effluent of AcoD. Also, in the rest of the reactors (i.e. R6-R7), the SARS-CoV-2 genes were not detected. Therefore, by increasing the temperature of the system the toxicity and effectiveness efficiency of intermediate metabolites increase over the virus and leading to severe viral particle reduction, and inactivation (Seruga et al., 2020; Sassi et al., 2018). Therefore, the application of higher OL in the thermophilic condition was ignored, since the OL of 1.5 gVS/L was enough to reduce the viral particles under a detectable level.

From Table 4, the presence of viral in the effluent of control samples (i.e. substrate + viral – inoculum), showed the impacts of different temperature states and OLs. However, the impact of temperature was more prominent over the OL. For example, in the psychrophilic temperature, the viral particles declined by 10–15 %, whereas in the mesophilic state viral decreased by 20–25 %. More particularly, in the thermophilic condition, the viral particles declined by 63.5 % without any fermentation process. Furthermore, in each temperature state, the viral particles showed minor reduction (~ 5–6 %) as the OL of systems increased, and this reduction could have been due to the entrapment of viral particles within the substrate. From Tables 3, and 4, it can be found that the AcoD process at different temperature and OL condition could eliminate the viral particles up to 99.7 % through (i) synergistic impacts of long operation condition and VFAs accumulation in psychrophilic temperature (ii) VFAs accumulation in mesophilic condition, and (iii) synergistic impacts of temperature and VFAs concentration in thermophilic condition.

4. Conclusion

In this study, the impacts of different operational temperature conditions, and OLs on the fate of SARS-CoV-2 in AcoD was evaluated. The results showed that the temperature as well as OL had synergistic effects on the viability of SARS-CoV-2 during AcoD. At psychrophilic temperature (20 °C) SARS-CoV-2 can survive until an OL of 3.5 gVS/L, whereas it was observed that the viral genes were reduced to undetectable level at mesophilic temperature (35 °C) with the same OL. Furthermore, a low OL of 1.5 gVS/L coupled with a high temperature of 50 °C is enough to reduce the viral genes under undetectable concentration. This study showed that the accumulation of intermediate metabolites (i.e. up to 2000 mg/L), along with the effective operational temperature can significantly reduce the viability of viral particles at the effluent of AcoD.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.pseps.2020.11.035.

Declaration of Competing Interest

The authors report no declarations of interest.

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