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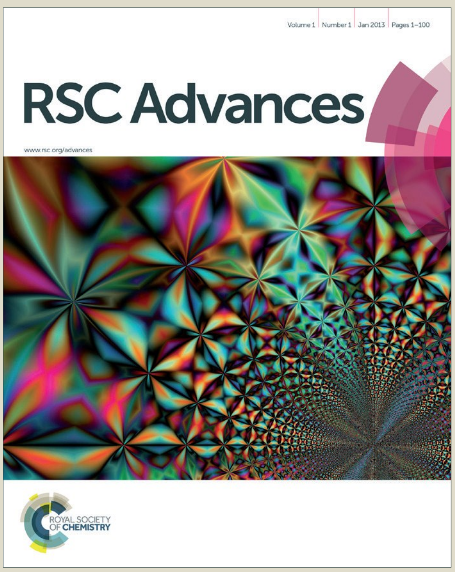

Anaerobic co-digestion of municipal wastewater sludge with food waste under different fat, oil, grease contents: study on reactor performance and extracellular polymeric substances

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Abstract

Linkage between reactor performance and microbial extracellular polymeric substances (EPS) was investigated in the 3 group semi-continuously mesophilic anaerobic co-digestion (ACoD) systems, treating municipal waste sludge (MWS) with food waste (FW) under different fat, oil and grease (FOG) contents. Addition of FOG to the test reactors enhanced the co-digestion process significantly in terms of the reactor performance and microbial activity. During the process, no major variations in pH and VFA/Alk were observed. Meanwhile, the biogas daily yield peaked at 810.3 mL/g VS_{added} when the FOG load was up to 42% of the volatile solids (VS), with the organic loading rate (OLR) of 5.2 g VS/L/d and hydraulic retention time (HRT) of 20 days. But excessive FOG load (55% on a VS basis) restricted biogas production by 40.3% when compared with the control unit (539.3 mL/g VS_{added}). At the end of digestion, 195 L, 381 L and 351 L cumulative biogas were obtained in 3 systems, respectively. Further analysis of extracellular polymeric substances (EPS) showed that the accumulation peaked at 648.5, 772.3 and 640.9 mg/L at the optimal digestion parameters, respectively. The proportion of LB-EPS were always less than that of TB-EPS, which accounted for about 40% and 60%. FOG enhanced systems (R2 and R3) obtained much higher EPS than control system (R1) for both humic acid substances (HS) and proteins (PN). Moreover, EPS variation revealed that 3 systems experienced the accommodation phase followed a vigorous phase and an exhausted phase along with elevated FOG adding. However, enhanced units may exhaust prematurely due to the “doping” phenomena.

Keywords: Anaerobic co-digestion; Food waste; Fat, oil, grease; Extracellular

polymeric substances.

1. Introduction

With the rapid development of cities construction, increasing numbers of wastewater treatment plants (WWTPs) have been setup and come into service for decades in China. As the main residual discharged from WWTPs, large amounts (30 million tons per annum) of municipal waste sludge (MWS) created a tremendous threat to the public health and environment when misconducted disposed.[1, 2] Anaerobic digestion (AD) has been evaluated as a promising biological technology to alleviate sludge disposal problem, since it converts organics in MWS to a renewable bioenergy resource in the form of methane.[3, 4] AD could be simultaneous beneficial with the sludge volume reduction, renewable energy recovery, potential hazardous compounds dilution and odor emission control, when compared with the conventional sludge disposal procedure.[5, 6] However, employing the MWS as a sole digestion substrate alone has been limited to the successful implementation of this approach due to the low C/N ratio contained in sewage sludge. This ratio in order of 6 to 16 is also regarded as a serious problem to the anaerobic digestion.[7] It should range from 20 to 30 in order to ensure sufficient nitrogen supply for cell production and the degradation of the carbon present in the process.[8]

Thus, applying the readily available high organic and excellent biodegradability waste, such as food waste (FW), is recognized as a desirable co-substrate material. Anaerobic co-digestion (ACoD) may substantially promote biogas yield due to the presence of abundant fat, oil, grease (FOG) in FW.[6] Despite the FOG has been frequently cited to effectively improve biogas production by 30% or more when directly add to the anaerobic digester based on their theoretical methane potential (1430 mL/g VS_{added}).[9, 10] Previous studies discussed the inhibition concerns or the potential for inhibition during ACoD. In fact, high FOG contents of organic wastes can lead to the accumulation of inhibitory compounds such as long chain fatty acids (LCFAs), resulting in process disturbances by affecting the microbial composition.[11, 12] LCFAs, the primary component of FOG presented in FW, are degraded anaerobically via the β-oxidation pathway to acetate and H_2, which are subsequently converted to methane. β-oxidation begins when the fatty acids are activated with coenzyme A and the subsequent oxidation leads to the release of acetyl-CoA and the formation of a fatty acid chain.[13] One concern is that LCFAs may have a detrimental effect on methanogenic bacteria when introduced at sufficiently high concentrations or loading rates. Researchers have suggested that the detrimental effect on methanogenic bacteria may be due to: sludge floatation and washout; transport limitation from bacteria being coated in a layer of LCFAs thereby hindering the cells access to substrates and tie ability to release biogas; or a LCFAs toxicity effect on methanogenic bacteria. Digester foaming is another operational concern associated with anaerobic digestion of lipids.[3] Foaming can result in inefficient gas recovery, inverse solids profile with higher solids concentrations at the top of a digester, blockages of gas mixing devices, fouling of gas collection pipes, and so on.[14] Hence, it is crucial to evaluate a practicable compromise between waste treatment capacity and biogas yield without causing operation instability.

AD occurs via four main steps, namely hydrolysis, acidogenesis, acetogenesis and methanogensis,[15] which is rely on a different microbial community with all of species living together in symbiotic associations. Therefore, it is necessary to clarify the different digestion parameters influencing community and optimize the microbial activity. However, most previous researches work on the microbial community diversity and dynamics, little information is available to specifically address the role of microbial metabolism in digestion process. Extracellular polymeric substances (EPS) of biological sludge treatment systems are a general property of a microbial community. The production and composition of EPS mainly come from bacterial active secretion, cell surface material shedding, cell lysis and desorption from the surface of an external matrix.[16] EPS is composed of a variety of organic substances including carbohydrates and proteins as the major constituents and humic substances, uronic acids and nucleic acids in smaller quantities.[17] It is noticeable that EPS partly results from microbial metabolism and thus is affected by the microbial community composition and its activity.[18] Additionally, resent suggestions have identified parameters, such as oil, grease, volatile fatty acid, detergents, proteins and products (EPS) from the metabolic activity of microorganisms as anaerobic digestion foaming causes.[19] A better insight into the degradation pathways and the by-products of these compounds during ACoD could provide additional information of the relationship of microbial response and digestion performance. Therefore, it is worth exploring whether FOG enhanced ACoD system affects the EPS accumulation.

This study investigated the influence of different FOG contents on ACoD of MWS and FW systems. Digestion process stability and general performance were discussed separately. The characteristics of microbial metabolism were discussed with EPS by detecting polysaccharides, proteins, humic acid substances and nucleic acids components. Further investigation is a matter of great interest not only in terms of deepening understanding of biological sludge treatment, but also in improving the efficiency of such treatment through the optimization of operational parameters.

2. Materials and methods

2.1 Inoculum and substrates preparation

Primary sludge and MWS were collected from a WWTP in Changsha, China. This plant located in the Xiang River region and annually disposes 280,000 tons of wastewater (90% domestic and 10% industrial sewage) with an oxidation ditch process. Raw food waste (RFW) was collected over 5 consecutive working days from a typical Chinese restaurant in Changsha, China. The collected samples were transported to laboratory within 1 h and stored at 4 °C for no more than 3 days.

Prior to pump into the digestion reactors: (i) primary sludge was thickened by gravity for 6 h at 4 °C with supernatant decant. The sediment seed sludge (SS) was sealed in a glass bottle with crimped butyl rubber stoppers and purged with nitrogen for 2 min to create an anaerobic condition. Bottles were subsequently incubated at 37 ^{o}C in a shaking incubator at 100 rpm for 15 days as an anaerobic adaptation period. (ii) RFW was squeezed excessively by cement compressor (YAW-300C, Hengda) followed Soxhlet extraction method [20] to eliminate FOG. (iii) MWS and FW were smashed into pasted respectively using an electrical food grinder (XTL-767, IFAVORITE. (iv) post-treated MWS, FW and FOG were stored at 4 ^{o}C until utilization. All of them were brought to room temperature before adding to the digester. The characteristics of SS and substrates are summarized in Table 1.

2.2 Reactor set-up and operation strategy

The semi-continuous experiments were performed under three strategies for a total progress of 120 days, using single stage mesophilic continuous stirred-tank reactors (CSTR) with 2.0 L working volume. Three group reactors were carried out in triplicates as $R1_{(1,2,3)}$, $R2_{(1,2,3)}$ and $R3_{(1,2,3)}$. 120 days operation were divided into four periods (30 d/period). Corresponding strategies are shown in Table 2. Each reactor initially inoculated 70% seed sludge and 30% co-substrates (MWS+FW). From the next day feeding was arranged for once a day. 100 mL (R1 and R2)/133 mL (R3) of digest materials were withdrawn each day and fed with the same volume to keep a constant HRT of 20 d/15 d. Co-substrates were mixed of MWS and FW with a TS ratio of 1:1. R1 group received only a mixture of co-substrates based on a percentage of the "safe" organic loading rate (OLR) at 3.0 g VS/L/d (g volatile solids per reactor volume per day) as a control, according to the optimized results of our preliminary assessment.[6] R2 group received a mixture of co-substrates as well as different FOG contents (4, 6, 8 and 10 mL in 4 period) as a test. R3 group received equivalent amounts of co-substrates and FOG contents as R2. But HRT of R3 (15 days) was shorter than R2 (20 days), in order to increase FOG load indirectly from the HRT aspect. The selection of FOG test contents and HRT was based on the fresh FW ingredients ratio (FW: FOG=1.7:1, m/m) and the fact that most of Chinese anaerobic digestion facilities are currently operated at a HRT of 15-18 days. Both in R2 and R3, the VS proportion of FOG to feeding were 33%, 42%, 49% and 55% for period I, II, III, and IV, respectively, as shown in Table 2.

All reactors were constantly mixed by magnetic stirrers (RW 205DS1, IKA Works, Inc., USA) at a uniform speed of 200 rpm and running program was set with 1 h on then 2 h off. Reactors were immersed and controlled at water bath within the range of 35 ± 1 ^{o}C using bolt electric heating rods, plastic film was also applied on the top to minimize heat loss. The pH value during this study was not adjusted. Effluent samples were carried out every 2 days for the subsequent anaerobic digestion performance and microbial EPS analysis. Biogas production was measured on a daily basis.

2.3 Digestion performance analysis

Feeding and effluent samples were centrifuged at 8000 g for 10 min, then the supernatant were filtered through disposable Millipore filter units (0.45 μm pore size) for the analysis of pH, volatile fatty acid (VFA), alkalinity (Alk), soluble chemical oxygen demands (sCOD) and total nitrogen. pH was determined using pH meter (Mettler Toledo FE 20). VFA, Alk, total solids (TS), volatile solids (VS), density, sCOD and total nitrogen were quantified according to the Standard Methods.[20] VFA were measured by titration with H_2SO_4. VFA = (volume (mL) of H_2SO_4 from pH 5.0 to pH 4.4 × 1.66) × 500. Alk represented as mg/L $CaCO_3$. All above sample analyses were performed in triplicate. Daily biogas from each reactors were collected with acidified water displacement method under standard conditions (25 ^{o}C, 1 atm). The composition of biogas (CH_4 and CO_2) was determined by a gas chromatograph (GC 2010, Shimadzu) using a thermal conductivity detector equipped with a 2 m × 3 mm stainless-steel column packed (Porapak Q, 80/100 mesh). Oven temperature was maintained at 40 ^{o}C during analysis. Injector and detector temperatures were 150 ^{o}C and 250 ^{o}C, respectively.

The results were reported at standard temperature and pressure (STP, 101.325 kPa, 273.15 K). The biogas production was reported as the volume of biogas produced per gram of VS_{added} (mL/g VS_{added}).

2.4 EPS extraction and analysis

EPS are composed of loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS) fractions based on the extraction methodology. The ingredients and quantities of EPS are strongly dependent on the sample source, the extraction process and the items of analysis conducted.[21] In this study, EPS extraction was carried out using a modified heating extraction method similar to that of Li et al.[22] Briefly, 35 mL digested sample was centrifuged (5810R, Eppendorf) at 8000 g for 10 min in order to remove the supernatant firstly. Without any delay, the residue was resuspend with preheated 50 ^{o}C PBS solution (0.01 mol/L, pH = 7.4) to original volume and vortex (Vortex-GenieH 2, Mo Bio) at 200 rpm for 1 min. The mixture was centrifuged at 8000 g for 15 min with the bulk solution and solid phase collected separately. The organic matter in the bulk solution was collected as LB-EPS. Next, the sludge pellet was rewash and resuspend to 35 mL again with aforementioned buffer solution and placed in a water bath at 60 ^{o}C for 30 min. The sludge mixture was centrifuged again at 8000 g for 15 min with sediment discarded. The organic matter in the bulk solution was considered as the TB-EPS. Both TB-EPS and LB-EPS were extracted in duplicates for each sample. After all the EPS fractions being extracted, 0.45 μm Millipore filter units were used to remove the particulates and low molecular weight metabolites. LB-EPS and TB-EPS contents were analysis immediately. In this study, the sum of the amounts of polysaccharides (PS), proteins (PN) and humic acid substances (HS) were used to represent the EPS. DNA was used to assess the extraction efficiency and quality by ranging from 2% to 15% of the total amount EPS during extraction.[23] PS in LB-EPS and TB-EPS were measured by the anthrone method with glucose as the standard.[21] PN were measured according to the Bradford reagent test kit. HS were measured by modified Lowry method with humic acid as the standard.[24] DNA were analyzed by the diphenylamine reagent colorimetric method using calf thymus DNA as the standard.[25] All above sample chemical analyses were conducted in triplicate and using chemicals of analytical grade. The results of assays were expressed as mean value ± standard deviation.

3. Results and discussion

3.1 Process stability

A widely acceptable pH condition, ranging from 6.5 to 7.5, is required for the ACoD process. Especially, methanogens are extremely sensitive to environmental pH and may exhaust under limit for a long time, making for the digestion system becomes acidification and irreversible with over-accumulated VFA. Fig. 1 shows pH in R1 remained almost constant equal to 7.5 during 120 days operation, whereas this was not the case for R2 and R3. The corresponding values dropped to 6.8 were noticed with FOG addition during period I and II, then recovered to the steady state levels without any artificial arrangement around 60 day. It might be that increased OLR presented in daily FOG addition resulted in slight acidification on acidogenesis stage. However, attributed to reactors acid-alkaline buffer, all systems showed a good acclimation to FOG condition spontaneously accompany with the digestion microbial community matured. Meanwhile, the pH fluctuation was associated with a marked variation in the VFA/Alk ratio, a reliable indicator of process stability. It is generally recognized that a stable unit is achieved when this ratio is less than 0.3-0.4. As can be easily seen from Fig. 1, after a short acclimation (1-10 d) to the mixed substrates, VFA/Alk in R1 decreased to 0.2 gradually around 40 day, which means a rather stable digestion system was established without any FOG addition. Moreover, this ratio practically maintained below 0.4 during the entire operation even on the start-up stage. The result revealed that ACoD system expressed higher process stability under lower OLR was also demonstrated by Fernández.[26]

Simultaneously, along with FOG adding to R2 and R3, a substantial increase in the VFA/Alk ratio was observed during period I. When FOG addition increased to 42% (on a VS basis), this ratio rapidly raised up to the peak values of 0.47 and 0.56 for R2 and R3 on day 31. These values close to the threshold indicated that poor system stability and an unfavorable balance between acidogenic and methanogenic microorganisms emerged, resulting in an acidifying digester. The nutrition balance condition with FOG addition depends on the faster hydrolysis and acidogenesis steps, which might generate large amounts of VFA and dropped in pH. Excessive VFA production can reportedly inhibit digestion process.[27] However, accumulated VFA were gradually utilized by predominant methanogens in the following days. This ratio was well above the limit range and dropped to 0.2 without artificial adjustment until day 60. Typically, this ratio in R3 was slight higher than R2 during day 1 to day 60. One of the reasons might be that shorter HRT in R3 induced the more washout of active methanogens in effluent removal then OLR increased indirectly when received equal load.

Fluctuation in pH and VFA/Alk ratio revealed that FOG might disturb systems stability, and a longer accommodation period was required (60 days in R2 and R3 >40 days in R1). On period III and IV (FOG VS % on 49% and 55%), no major variations in pH and VFA/Alk may explained by the fact that the FOG load in this study was acceptable and a higher treatment potential could be explored.

3.2 General performance

Based on the experimental results from Table 3, the trend of VS concentrations were very similar to that of sCOD. VS and sCOD contents showed minor changes in R1 control digester when received MWS+FW only. VS and sCOD concentrations in R2 and R3 reached peaks when FOG adding up to 42% (VS %). However, with a 60 days adaptation, VS and sCOD in R2 and R3 dropped to R1 levels in period III and IV accompany with the optimal operation of the digester. The data proved that the readily decayed solid organic materials were rapidly degraded by the microorganisms, as it can be also demonstrated by the elevated VS/TS ratio. VS and sCOD concentrations in R3 were practically lower than that of R2. By taking into account that the two experimental systems operated at the same OLR, it is anticipated that shorter HRT (R3) outflowed much more VS in effluent.

Furthermore, the final percentage of sCOD removal efficiency in R2 and R3 reached 77% and 75% when compared with 61% on R1. Two FOG enhanced digestions obtained higher sCOD removal rates dramatically, which were consistent with the other studies reported that high-organic materials have a positive effect on co-digestion process.[28] These findings proved that co-digestion with FOG and longer HRT have more advantages in organic conversion, probably due to a balanced nutrient ratio with the mixed substrates and enhanced pH buffering capacity.

3.3 Biogas production

Appling the MWS as the mono-substrate has been limited to the successful implementation of biogas production due to the low C/N ratio contained in sewage sludge. This ratio in order of 6 to 16 (w/w) is regarded as a serious problem to the anaerobic digestion. It should range from 20 to 30 in order to ensure sufficient nitrogen supply for cell production and the degradation of the carbon present in the process. Fig. 2 (a) presents the evolution of biogas daily production (g^{-1} VS_{added}) in 3 digestion units during 4 periods. The averaged biogas production in R1 equal to 540 mL/g VS_{added} as a control, and the corresponding C/N ration in co-substrate reached 21.9. In comparison, biogas production in two test units apparently fluctuated when received a mixture of elevated FOG contents. Along with FOG addition from 33% to 42% (VS %), biogas yield peaks (about 862 and 715 mL/g VS_{added}) were achieved for R2 and R3 around day 44 and day 40. More specifically, the co-digestion of FOG at 42% (VS %) and OLR at 5.2 g VS/L/d with HRT up to 20 days resulted in biogas yield promoted by 45%, which demonstrated that a delicate balance was achieved between the rates of hydrolysis/acidogenesis and methanogensis. However, a remarkable biogas production downhill was observed in two test units around day 60. Subsequently a reduction below R1 level at day 80 was recorded. The further increased FOG addition exerted a negative effect on the biogas yield. Biogas production were practically inhibited by 37.7% and

40.3% for R2 and R3 during period IV with FOG progressively increased to 55% (VS %), only 336.2 and 321.8 mL/g VS_{added} was achieved. Thus, FOG addition at 42% (VS %) and an OLR of 5.2 g VS/L/d was found to be optimum for the maximal waste treatment capacity while still maximizing biogas yield from the process. The greater biogas output in the FOG enhanced process were also reported by Davidsson and Luostarinen.[9, 29] However, according to Luostarinen et al.,[29] an upper limited content (to the order of 55% on a VS basis) of FOG degradation is incomplete and biogas yield decreased. Martinez et al. also found that treating a mixture with a higher content of lipid-rich waste resulted in a decrease in specific methane production, although an adaptation period was applied to the reactor.[30] Another reason why a lower gas yield obtained is the adsorption of FOG components onto sludge, which then would have precluded degradation by microorganisms.[31]

Moreover, Fig. 2 (b) presents the cumulative biogas in R1 during 120 days process was about 195 L. R2 and R3 were much higher than R1 level, 381 L and 351 L were obtained respectively. The promotion ratio of 95% (381 vs. 195 L) and 80% (351 vs. 195 L) was similar to the mean value of relative OLR (85%) in 4 periods, which proved that organic loading presented in FOG significantly attribute to biogas production. In addition, a more efficient organic material conversion in R2 (95%) than R3 (80%) was also in accordance with the aforementioned performance analysis. It was likely that the increase in HRT would drive the performance improvements.

Specifically, a higher biogas production (g^{-1} VS_{added}) in R3 than R2 about 11.6% (averaged 547 vs. 490 mL/g VS_{added}) was noted during initial feeding stage (1-14 d). It was possible that R3 with a shorter HRT increased OLR indirectly, resulted in more biogas was harvested in temporary.

Martinez et al. also reported an increase in biogas production with the decrease in HRT (increasing organic loading rate). This behavior may be rationalized by the low complex nature of the substrate, which allowed for its rapid conversion.[31]

After then, R2 turned over from day 14 and cumulative biogas ascended from day 24, as black pointed out in Fig. 2. It was anticipated that a vigorous digestion system may prematurely exhaust and induce a reduction in biogas production, which was considered as a “doping”.

Furthermore, the evolution of biogas proportion in 3 reactors was characterized by GC. As revealed in Fig. 3, it was clear that CH_4 dominated in biogas after co-digestion especially for the FOG addition systems, which increased to average 70.9% and 66.4% for R2 and R3. It was concluded that adaptation of biomass to FOG content was a rather gradual process. The enhanced biogas conversion efficiency supported that FOG had positive effects on the hydrolysis rate and methane potential, which were attributed to well-functioning methanogens, scavenging the organic acids formed by acidogenic bacteria.[32]

3.4 Extracellular polymeric substances (EPS) variation

3.4.1 Accumulation of total EPS

Alterations a proportion of digester feed for FOG encouraged the activity of different microbial populations in the digestion systems, allowing the impact on EPS products and digestion performance to be assessed under controlled conditions. The difference of total EPS (LB-EPS and TB-EPS) were presented in Fig. 4. Total EPS contents in 3 reactors showed a similar pattern which was described as an “n” shape. This process was divided into three phase, the accommodation phase (1-20 d) followed a vigorous phase (21-70 d) and an exhausted phase (71-120 d) along with running.

At the beginning (1-20 d) of feeding with different digestion substrates, a large decrease of total EPS was observed. The decreased EPS fractions in 3 reactors probably attributed to the fact that the microorganisms are facing a sudden stepwise increase of organic loading, they overproduce hydrogen due to this change and afterwards they re-adjust their operation based on the newly applied conditions slowing down their metabolic operations. At the end of the accommodation stage (20-30 d), each system arrived at EPS accumulation vigorous phase gradually from day 20 to day 70 due to the major stimulation of the active digested microbial population against loading impact. These results seemed to confirm that microorganisms were progressively acclimated to the new co-substrates, which were demonstrated in the forehead performance analysis.

Specifically, with adding FOG to the two test units, a more remarkable EPS accumulation was obtained. R2 and R3 reached EPS plateau stage in advance and stayed longer (around 30-60 d) when compared with R1 (day 65 only). From the whole 120 days operation, total EPS concentration in test units was found to be increased by 19.4% and 5.2% for R2 and R3 in this study, averaged 503.6 and 443.9 vs. 422.0 mg/L. EPS concentration peaked at day 39 (772.3 mg/L), day 55 (640.9 mg/L) and day 67 (648.5 mg/L) for R2, R3 and R1 successively. In fact, anaerobic digestion with high loading is liable to cause the accumulation of soluble microbial products.[33] Because there are abundant microbial residuals in organic waste digestate following digestion, bring about the possibility of microbial EPS accumulation.[34] Based on the operation mode of CSTR, same volume of digestion material were withdrawn and fed to keep a constant working volume of 2.0 L in each reactor. The effluent digestion samples collected were mutually independent. In this study, the substrates degradation and the microbe release are regarded as the 2 major sources of EPS contents. In fact, EPS variation from the substrates aspect was controlled identically by receiving the equivalent amounts of co-substrate (MWS+FW at 3.0 g VS/L/d) in all reactors. Thus, the fluctuation of EPS contents can be attributed to the cell lysis in active microbial population with response to FOG. The released intracellular organics which benefited microbial reproduction and produced more EPS in FOG enhanced systems. Ng et al. [35] also reported that biomass undergone endogenous respiration and cell lysis would occur at longer mean cell residence times, which would cause the further release of EPS into the bulk solution. Besides, R2 produced more EPS with a longer HRT than R3. Many researchers have found that the EPS in various microbial aggregates increases with an increasing HRT.[22] In addition, the sCOD loading also has a significant effect on the EPS accumulation.[33] The relatively longer HRT in R2 and higher sCOD loading in the R2 and R3 (1,300-1,500 mg/L) might generate more EPS than that at a lower sCOD loading in R1 (550 mg/L). Hence, the specific increase in the R2 and R3 EPS were reasonable.

It was interesting to note that unlike stable biogas yield condition, an EPS reduction in R1 from day 60 was observed without FOG interference. FOG enhanced units of R2 and R3 even declined below the control unit around day 70. It could be inferred that a long-term running (over 60 days) digestion unit might produce large amounts of toxic metabolic products then restrained bacterium to generate EPS. Enhanced digestion systems may exhaust prematurely due to the flush metabolism accumulation, which was described as a "doping" phenomena before.

Nevertheless, the direct toxicity of excessive FOG on digestion microbial community and the change of microbial metabolism also should be taken into consideration. In fact, high FOG contents of organic wastes can lead to the accumulation of inhibitory compounds such as long chain fatty acids (LCFAs). It is well know that the accumulation of LCFAs may inhibit anaerobic digestion because of their direct toxicity toward acetogens and methanogens, the two main groups involved in LCFA breakdown.[36] Another inhibiting mechanism is the adsorption of surface active acids onto the cell wall,[37] which affects the processes of transportation and protection. As Sutherland et al. [38] pointed out that the aggregations of microorganisms can provide EPS as energy supplication and protective layer for the cells to against toxic substances damage from harsh external environment. With the FOG content increasing, the products of EPS were able to combine with LCFAs then greatly stimulated due to the protective response of the microorganism.

3.4.2 Loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS)

The structure of microbial EPS is generally subdivided by a two layer model.[39] The inner layer is constitutive of tightly bound EPS (TB-EPS), which have a certain shape and are bound tightly and stably with the cell surface. The outer layer, which consist of loosely bound EPS (LB-EPS), are a loose and dispersible slime layer without an obvious edge.

Fig. 4 depicted that the proportion of LB-EPS in microbial aggregates were always less than that of TB-EPS, which accounted for about 40% (LB-EPS) and 60% (TB-EPS) in 3 reactors during 120 days operation. Such a discrepancy may be attributed to harsh parameters in TB-EPS extraction procedure employed by heating in 60 ^{o}C water bath. Another reason might be that the loosely binding existed in LB-EPS, led to readily fluctuation during digestion process. The LB-EPS in sludge flocs would acting as the primary surface for cell attachment and sludge flocculation. In a recent study, the LB-EPS content were found to be more closely related to the performance of microbial activity, while no obviously correlation could be found between the TB-EPS concentration and the microbial aggregates.[22]

3.4.3 Subfractions of EPS

EPS are essentially a mixture of biomolecules, which can be treated as “soft matter” and their subfractions can be largely affected by the digestion operating conditions. EPS are comprised of a wide variety of organic compounds including polysaccharides, proteins, humic acid substances, DNAs (DNA), and so on.[40] The different EPS subfractions extracted from 3 systems were shown in Fig. 5.

For all 3 reactors, R2 and R3 obtained much higher EPS concentrations than R1 for both humic acid substances (HS) and proteins (PN). In addition, HS were the predominant components at the corresponding concentrations of 194, 254 and 221 mg/L, followed by PN at 110, 141 and 130 mg/L for R1, R2 and R3, respectively. PS were dispersed evenly, which accounted for a small but stable proportion to EPS amounts at 20-30% (in supporting info). It was noted that in the subfractions, a similar cumulative peaks of HS took place in 3 reactors which were in accordance with the total EPS variation feature as revealed in Fig. 4. These finding also indicated that the relative contribution of cumulative EPS were mainly attributed to the HS components. One concern is that there are many humic like substances produced from the degradation of feeding substrates. In studying primary sludge digestion, Miron et al.[41] reported that the hydrolysis of lipids and carbohydrates increased with increasing solid retention time, whereas protein hydrolysis only occurred under methanogenic conditions.

According to previous reports, EPS plays an important role in microbial adhesion and aggregation processes, promoting to the formation and stability of microbial community structure.[16, 42] Each EPS fraction contains different components and represents rather distinct chemical property. The polysaccharide and protein in TB-EPS are independent of the influent carbon source and C/N ratio.[43, 44] However, the protein content and the carbohydrate content in LB-EPS are related to the influent C/N ratio.[44] Such differences indicated that the different EPS fraction had different components.[45]

3.4.4 Change degree of EPS subfractions

Considering the dedicate LB-EPS and TB-EPS subfractions characteristics inferred by ACoD process largely depend on not only concentration but also fluctuation degree. Consequently, 2 mathematical parameters (p and k value) were involved in to describe the change degree of different subfractions so as to better understand the complicated evolution in this study. (The detailed results were presented in the Supplementary

Information).

The p value was determined as follows:

$$\mathrm{p}_i^{a,b,c} = \frac{\mathrm{Cn}_{i+1}^{a,b,c} - \mathrm{Cn}_i^{a,b,c}}{\mathrm{Cn}_1^{a,b,c}} \text{ , i=1, 2, 3… 119;} \quad \mathrm{p}_0^{a,b,c} = 0$$

where Cn represented EPS fraction concentration on the each day; a, b and c represented PS, PN and HS, respectively.

Then, the indicator k was given by the absolute value of p together. The k value was expressed as follows:

$$\mathrm{k}^{a,b,c} = \sum_{i=1}^{n=119} |\mathrm{p}_i^{a,b,c}|$$

A higher k value represented the increased EPS fluctuation degree. As can be seen in Fig. 6, the k value disparity of PS and HS in TB-EPS were less significant than that in LB-EPS, as black and grey oval pointed out. Besides, these findings also suggested that the degree of change in LB-EPS subfractions appeared to be more obvious than that in TB-EPS. Although the metabolism were considered capable of dissolving bound EPS to the supernatant in the meantime, the released TB-EPS from the inner cells were hard to diffuse out of the sludge. The variation of LB-EPS observed in the this study under FOG conditions was anticipated to be related more directly to different levels of microbial EPS secretion as active responses to external environmental challenges.

Furthermore, compared to the variations in EPS subfractions with the changes in process condition, the extent of the changes in PN was the most remarkable. The k values of PN were dramatically higher than other subfractions and there was a trend of change in correlation with the operational condition, as red arrow pointed out. PN are believed to play a crucial role in the structure, properties and functions of sludge aggregates.[46] The variation in the PN concentration might be attributed to the presence of a large quantity of exoenzymes, as suggested by Frølund et al.[24] The easy degradation and uptake of readily biodegradable organic substrates, such as glucose and acetate, gives rise to a high level of exoenzymes in the EPS matrix.[47] The higher k value of PN rather than any subfractions proved that the substrates arising from the digested materials were readily biodegradable.

4. Conclusions

Mesophilic co-digestion of MWS with FW under proper FOG conditions led to substrates better balanced and efficiently degradable. Biogas production and COD reduction were enhanced significantly in the FOG test systems. But excessive FOG addition disturbed process stability and restricted digestion performance. EPS variation revealed that the microbial activity was affected by FOG. Each EPS subfractions play different roles in microbial metabolize activities due to the distinct chemical property. EPS analysis also indicated that the FOG enhanced systems may exhaust prematurely due to the “doping” phenomena. In general, the complexity and extent of synergic interactions in the microbial world during ACoD is greatly unexplored and further research requires an essential step towards optimizing the digestion performance.

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Figure captions

Fig. 1 pH and VFA/Alk ratio in three group anaerobic co-digestion reactors during 120 days operation.

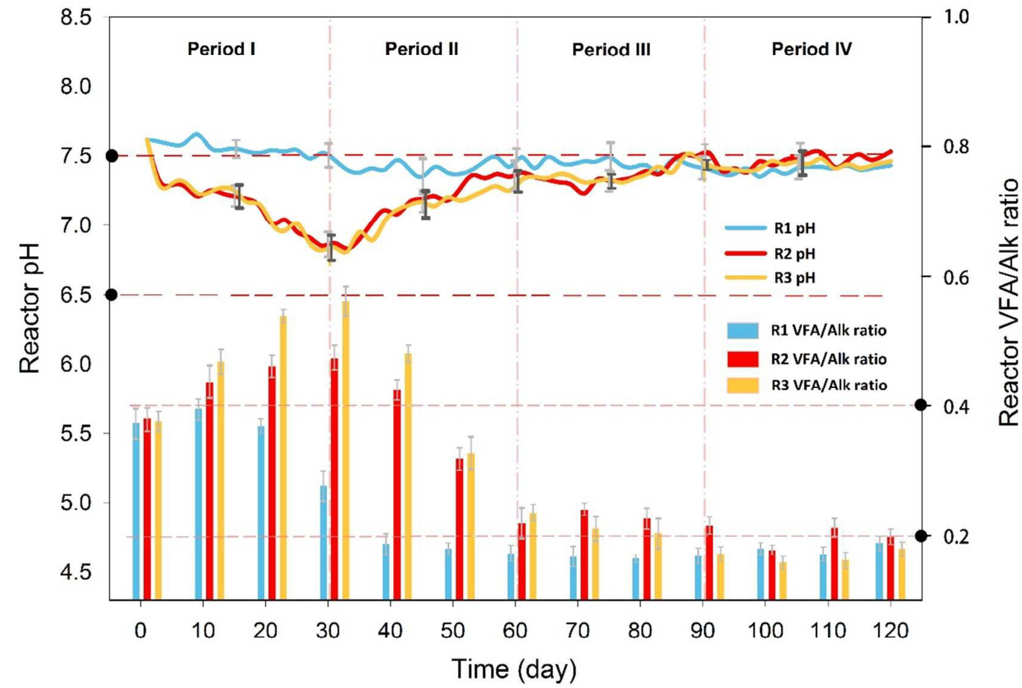
Fig. 2 Daily biogas per VS_{added} production (a) and cumulative biogas (b) production in three group anaerobic co-digestion reactors during 120 days operation.

Fig. 3 Evolution of biogas proportion after anaerobic co-digestion with different operation strategies.

Fig. 4 Cumulative EPS concentrations and the proportion of LB-EPS and TB-EPS in three group anaerobic co-digestion reactors during 120 days operation.

Fig. 5 Heatmap of EPS subfractions in three group anaerobic co-digestion reactors during 120 days operation.

Fig. 6 Change degree of LB-EPS and TB-EPS subfractions in three group anaerobic co-digestion systems, which was described by k value.



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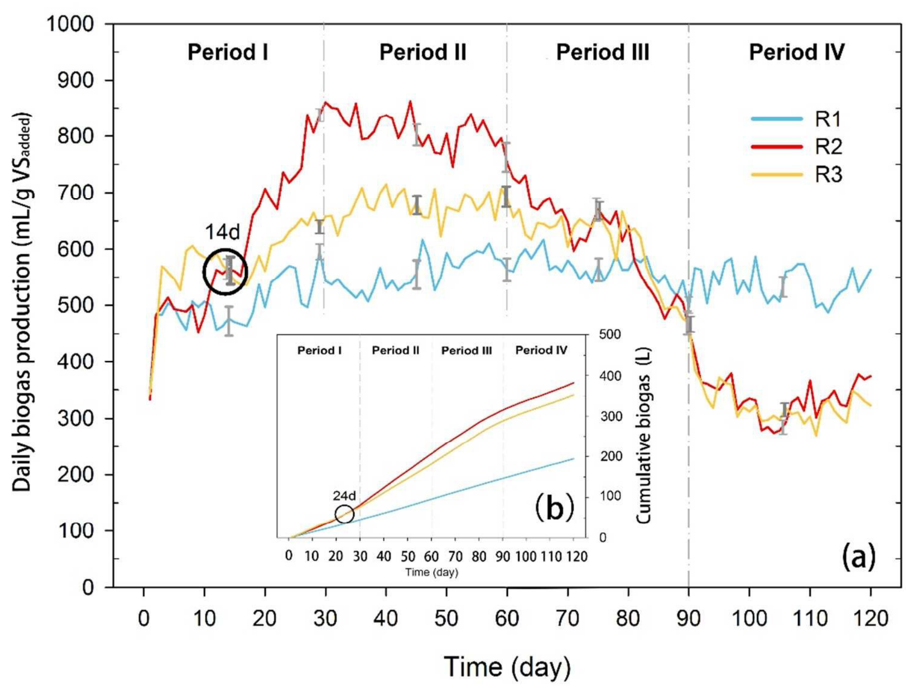

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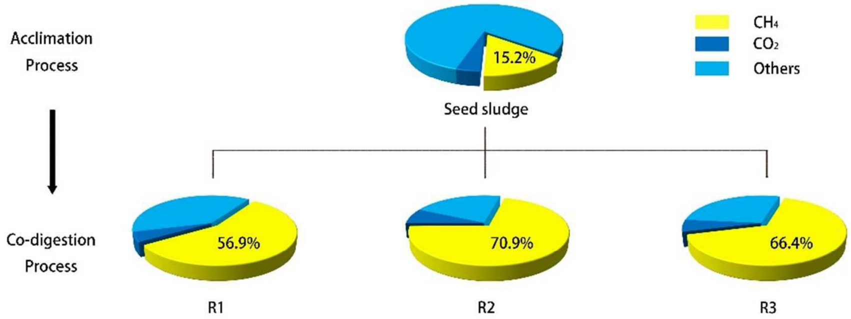

Fig. 3 Evolution of biogas proportion after anaerobic co-digestion with different operation strategies.

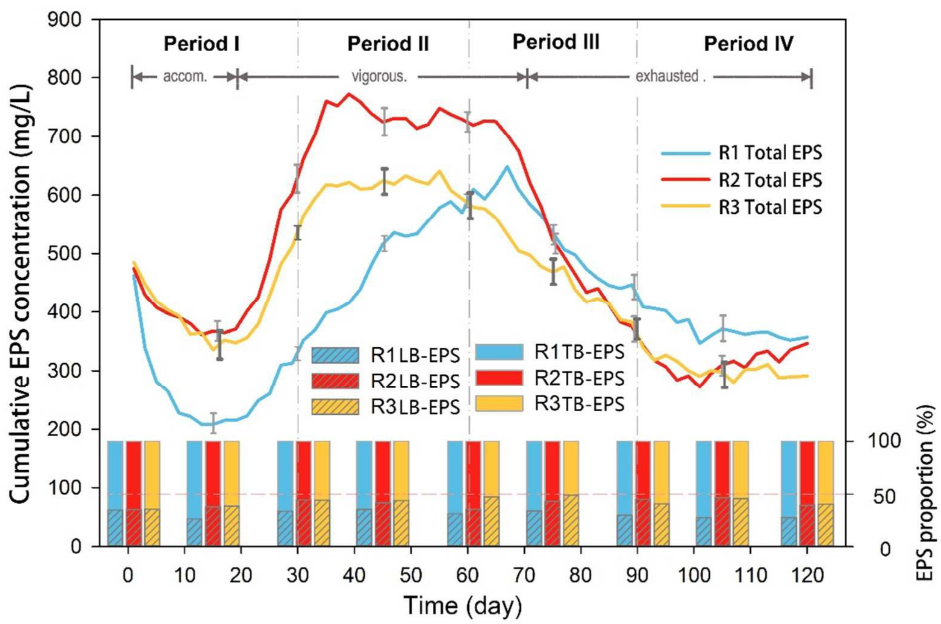

Fig. 4 Cumulative EPS concentrations and the proportion of LB-EPS and TB-EPS in three group anaerobic co-digestion reactors during 120 days operation.

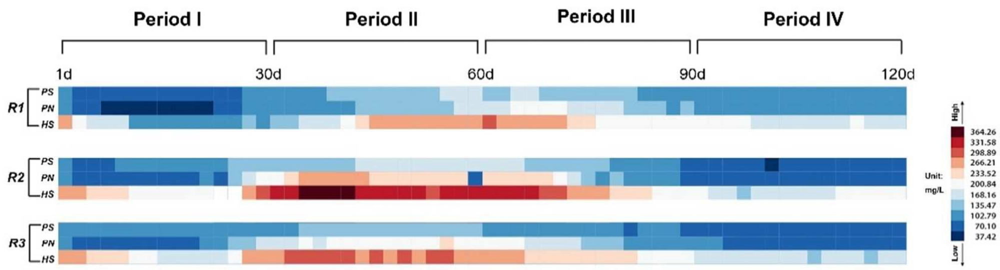

Fig. 5 Heatmap of EPS subfractions in three group anaerobic co-digestion reactors during 120 days operation.

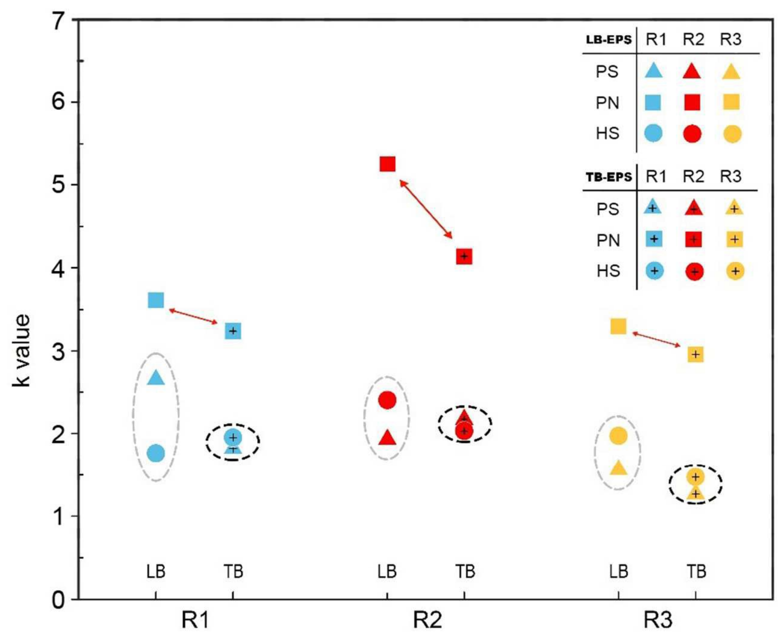

Fig. 6 Change degree of LB-EPS and TB-EPS subfractions in three group anaerobic co-digestion systems, which was described by k value.

Table List

Table 1

Characteristics of the seed sludge and feed substrates in co-digestion experiments.

Item	Type of raw materials				
	SS	MWS	FW	CoSub	FOG
Density (g/mL)	0.9 ± 0.1	1.2 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	0.9 ± 0.1
pH	7.6 ± 0.1	7.4 ± 0.1	4.6 ± 0.1	6.3 ± 0.1	4.3 ± 0.1
sCOD (g/L)	0.4 ± 0.5	17.9 ± 10	103.1 ± 20	51.1 ± 20	123.0 ± 20
Moisture (%)	95.4 ± 0.1	75.0 ± 0.1	60.9 ± 0.1	69.5 ± 0.1	21.3 ± 0.1
TS (g/L substrate)	40.6 ± 0.2	289.9 ± 0.2	402.4 ± 0.2	333.7 ± 0.2	724.5 ± 0.2
VS (g/L substrate)	13.3 ± 0.2	113.6 ± 0.2	389.7 ± 0.2	221.2 ± 0.2	718.0 ± 0.2
VS/TS (%)	31.0 ± 0.2	39.0 ± 0.2	96.0 ± 0.2	61.2 ± 0.2	99.0 ± 0.2
VFA (mg/L)	617.8 ± 0.5	5118.2 ± 0.5	2965.0 ± 0.5	4278 ± 0.5	6371.2 ± 0.5
Alkalinity (mg/L as $CaCO_3$)	1645.8 ± 0.5	736.7 ± 0.5	2664.6 ± 0.5	1488 ± 0.5	1136.4 ± 0.5
C/N (w/w)	5.6 ± 1.0	7.4 ± 1.0	37 ± 1.0	21.9 ± 1.0	15 ± 1.0

SS = Seed sludge; MWS = Municipal waste sludge; FW = Food waste; CoSub = co-substrates of municipal waste sludge with food waste at TS ratio of 1:1; FOG = Fat, oil, grease; VFA = volatile fatty acid.

Table 2

Operation strategies in three group mesophilic anaerobic co-digestion reactors.

Period	Group	Substrate	HRT	OLR	FOG %	Relative loading
		Units	(d)	(g VS/L/d)	(VS %)	(%)
	$R1_{(1,2,3)}$	CoSub	20	3.0	0	100
I (1-30d)	$R2_{(1,2,3)}$	CoSub +FOG (4 ml)	20	4.5	33	150
	$R3_{(1,2,3)}$	CoSub +FOG (4 ml)	15	4.5	33	150
	$R1_{(1,2,3)}$	CoSub	20	3.0	0	100
II (31-60d)	$R2_{(1,2,3)}$	CoSub +FOG (6 ml)	20	5.2	42	170
	$R3_{(1,2,3)}$	CoSub +FOG (6 ml)	15	5.2	42	170
	$R1_{(1,2,3)}$	CoSub	20	3.0	0	100
III (61-90d)	$R2_{(1,2,3)}$	CoSub +FOG (8 ml)	20	5.9	49	200
	$R3_{(1,2,3)}$	CoSub +FOG (8 ml)	15	5.9	49	200
	$R1_{(1,2,3)}$	CoSub	20	3.0	0	100
IV (91-120d)	$R2_{(1,2,3)}$	CoSub +FOG (10 ml)	20	6.7	55	220
	$R3_{(1,2,3)}$	CoSub +FOG (10 ml)	15	6.7	55	220

CoSub = co-substrates of municipal waste sludge with food waste (none FOG contents); FOG = fat, oil, grease; OLR = organic loading rate. Each reactor was paralleled in triplicates.

Table 3

Experimental results of the anaerobic co-digestion during 120d process.

Parameter	Period I			Period II			Period III			Period IV		
	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3
VS (g/L)	15.6 ± 0.7	20.1 ± 0.7	17.4 ± 0.3	16.1± 0.3	21.2 ± 0.7	19.6 ± 0.4	16.4 ± 0.3	18.8 ± 0.3	16.7 ± 0.2	15.6 ± 0.2	16.9 ± 0.2	14.9 ± 0.2
VS/TS (%)	36.9 ± 1.4	46.3 ± 1.5	44.3 ± 1.4	43.2 ± 1.9	53.0 ± 1.7	54.8 ± 1.6	48.8 ± 1.1	55.1 ± 1.9	51.8 ± 1.3	53.5 ± 1.3	54.0 ± 2.0	55.9 ± 1.8
sCOD (mg/L)	440.2 ± 13.2	1257.6 ± 31.5	1148.0 ± 27.7	553.8 ± 11.5	1533.5 ± 31.2	1302.1 ± 20.8	550.2 ± 11.9	725.6 ± 12.0	718.1 ± 9.3	552.2 ±9.7	699.4 ± 8.4	689.8 ± 9.6
sCOD reduction (%)	61.8 ± 1.7	67.7 ± 1.9	63.8 ± 2.2	60.2 ± 2.4	69.6 ± 1.8	64.4 ± 2.0	61.0 ± 1.3	74.2 ± 1.9	70.9 ± 1.9	61.5 ± 1.7	77.1 ± 2.1	75.7 ± 2.2
Biogas yield (mL/d)	1504 ± 20.8	2698 ± 33.6	2552 ± 37.5	1672 ± 21.0	4181 ± 34.6	3493 ± 29.3	1690 ± 17.6	3604 ± 31.7	3550 ± 29.6	1618 ± 16.8	2219 ± 18.3	2123 ± 19.7
Biogas yield (mL/g VS_{added})	501.3 ± 6.9	611.3 ± 7.5	578.5 ± 8.4	557.5 ± 7.1	810.3 ± 6.7	677.2 ± 5.7	563.6 ± 5.9	613.0 ± 5.4	603.8 ± 5.0	539.3 ± 5.6	336.2 ± 2.8	321.8 ± 2.9
Biogas promotion (%)	—	21.9 ± 7.1	15.4 ± 7.3	—	45.3 ± 6.8	21.5 ± 5.9	—	8.8 ± 5.9	7.1 ± 5.3	—	-37.7 ± 4.7	-40.3 ± 4.9

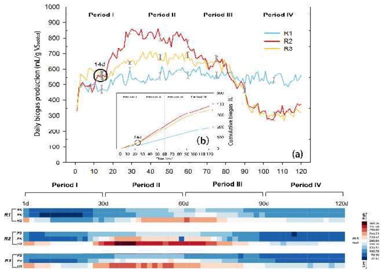

Table of contents:

Relationship of extracellular polymeric substances and microbial activity were investigated in 3 group fat, oil, grease (FOG) enhanced ACoD reactors.