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The effect of ISR on OFMSW during acidogenic fermentation for the production of AD precursor: kinetics and synergies

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Acidogenic fermentation of the organic fraction of municipal solid waste (OFMSW) and its components (food waste, paper waste) was studied in a batch percolator reactor without artificial pH adjustment. The effect of inoculum to substrate ratio on process performance, in terms of pH, hydrolysis and volatile fatty acid (VFA) production, has been investigated. The inoculum to substrate ratio (ISR) was varied from 0 to 0.36 VS/VS and at optimized conditions for fermentation of OFMSW, with ISR 0.23, pH, hydrolysis and acidification yield were 5.5, 625 mg sCOD per g BD VS and 408 mg g⁻¹ BD VS respectively. Due to the uplift of pH from 4 to 5.5 because of addition of ISR, the VFA composition was dominated mostly by butyric, acetic acids and propionic acid. Kinetics regarding rate of hydrolysis and acidification were calculated and reported. A significant synergistic effect was noticed in the acidification and hydrolysis, which were 1.76 and 1.35 fold higher than individual components (paper waste and food waste) of OFMSW, respectively and approximately 70% of biodegradable solid carbon solubilized into the liquid carbon within a short retention time of 78 h.

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1. Introduction

The organic fraction of municipal solid waste (OFMSW) is currently one of the major interests in the research community due to its high biodegradability (BD) and its potential for environmental pollution.¹ Especially in developing countries, OFMSW is characterized by a wide range of easily biodegradable organic matter which includes 37–51% carbon and 80–90% volatile solids.² Food waste (FW) and paper waste (PW) are two major components of OFMSW, comprising a total of *ca.* 90%.³ Simultaneously the nature of these wastes differ to a greater extent towards acidification. While PW contains a large amount of carbohydrate, it has a high C/N ratio which corresponds to the lack of basic raw materials for the production of amino acids for microorganisms.⁴ Conversely, food waste (FW) is nitrogen-rich due to the abundant availability of protein.⁵ Therefore, the supply of essential nitrogen element from FW can enhance the acidogenic fermentation of OFMSW. However, in municipal waste, PW and FW are always mixed.

Moreover, MSW with high organic fraction is a potential resource, if treated under controlled conditions.^{6,7} From this perspective, volatile fatty acids (VFA), generated *via* anaerobic fermentation of OFMSW, is considered as an economical and feasible technology practice, since these VFAs have a broad range

of practical applications which includes methane recovery from anaerobic digestion, biological nutrient removal (BNR) in modern municipal and industrial wastewater treatment plants (WWTP), polyhydroxyalkanoates (PHA) production.^{8–10} The composition and concentration of these VFAs are influenced by several factors, including hydraulic retention time (HRT), pH, initial organic load (IOL), inoculum to substrate ratio (ISR), C/N ratio, particle size and reactor modes.^{6,11–14}

Most of the studies were reported for enhancement of VFA production through adjustment of pH by adding different chemicals. However, it is not economically viable at large-scale applications. Moreover, disposal of bioreactors effluent with high concentration of chemicals may lead to high cost and can have negative impacts on bacterial community.¹³ Some studies reported that VFAs could be efficiently produced without artificially controlling the pH.^{15,16} The pH can be elevated by applying different processing methods such as ultrasonication pretreatment,¹⁷ change in temperature 45–70 °C,^{18,19} ISR 0.2–1 (ref. 20) and mixed fermentation.^{21,22} Among all, ISR and mixed fermentation methods are recognized for their practical applicability at the large-scale for the separation of biodegradable dissolved organic matter as a renewable carbon sources for AD precursor.

ISR (microorganism) is the fundamental driver to accelerate the acidogenic process. The inoculum type and its quantity are significant factors that affect the hydrolysis and acidification pathways.²³ Most of the research studies reported in the literature investigated the significance of ISR during acidogenic fermentation of FW with pH controlled by external means^{12,24} Furthermore, a wide range of ISR values were employed in the existing studies, however, it is quite complicated to compare

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different substrates and inoculum used. For example, an ISR 1 was suggested for the acidogenic fermentation of grass,²⁵ 0.02 for manure,²⁶ 0.13 for FW²⁰ and 0.5 for mixed fermentation of excess sludge and FW.²¹ Several studies have been conducted for VFA production from sole FW.^{6,11,13,21,22,27–29} Sole PW was used for the production of methane³⁰ and ethanol production at a controlled pH 5.³¹

However, particular organic components of OFMSW can significantly influence its behavior during anaerobic fermentation. To the best of our knowledge, no study has been conducted to compare the distinction in product characteristics from mixed OFMSW and its sole components (*e.g.* food waste and paper waste) during the acidogenic fermentation. The results will help to explain the effects of individual fraction of OFMSW on the acidification.

In this study, a series of ISR, namely 0.09, 0.23 and 0.36, were applied for VFAs fermentation of mixed OFMSW in percolator reactor, with respect to the comprehensive evolution of products characteristic and their kinetics. The novel aspect of this study was to investigate mixed- and mono-fermentation from OFMSW without pH control, as well as their synergistic effects, based on sole components, were evaluated for hydrolysis and acidogenic stages, respectively. The results obtained above were used to discover the possible pH mechanisms and relative parameters for enhancing acidogenic fermentation of OFMSW.

2. Materials and methods

2.1. Substrate & inoculum

A physical representative mixture of OFMSW was prepared on the basis of composition of the typical municipal solid waste (MSW) of China.³ The representative FW was prepared on the basis of the data reported by Xu *et al.*,³² which were mediated over the year of investigation. Before loading into the percolator, the synthetic OFMSW was manually shredded to maintain a uniform size of 40–50 mm (FW) and 5 × 20 mm (*L* × *W*) paper pieces before being mixed. Anaerobically digested sludge under mesophilic conditions was obtained from the Dongcun waste treatment plant, Beijing, China and after natural sedimentation for 15 days was used as inoculum. Additionally, the sludge was sieved through a 2 mm sieve for removal of large particles. Composition and ultimate analysis are shown in Tables 1 and 2.

2.2. Percolator reactor and experimental setup

The laboratory scale percolation reactor consisting of a rectangular tank with a total volume of 30 L and working volume 20 L, as shown in Fig. 1, was used in the study.

Table 1 Composition and characteristics of OFMSW and inoculum^a

Component	TS%	VS%	Dry COD	BD (VS%)
FW	94.52 ± 3.1	94.52 ± 2.9	1.33 ± 0.01	72.3 ± 4.12
PW	96.46 ± 2.1	79.66 ± 3.2	1.008 ± 0.053	60.2 ± 3.02
OFMSW	27.78 ± 1.1	87.3 ± 3.9	1.175 ± 0.04	69.37 ± 4.5
AD sludge	6.45 ± 0.4	40.3 ± 2.4	0.43 ± 0.06	15.2 ± 0.41

^a Mixing on the basis of wet weight.

Table 2 Ultimate and component analysis of substrate and inoculum

Component/element	Ultimate analysis (%)					Component analysis (%)				
	Carbon	Hydrogen	Nitrogen	Oxygen	Sulphur	TKN	Crude lipid	Crude protein	Crude carbohydrate	Crude fiber
Food waste	47.05 ± 0.26	6.58 ± 0.29	2.869 ± 0.2	37.72 ± 1.6	0.27 ± 0.015	3.09 ± 0.2	4.74 ± 0.13	19.33 ± 0.81	12.5 ± 0.41	58.43 ± 1.7
Paper waste	33.15 ± 0.5	4.89 ± 0.08	0	41.67 ± 0.1	0.042 ± 0.04	0	1.89 ± 0.07	0	0	76.3 ± 2.3
OFMSW	40.31 ± 0.31	5.76 ± 0.12	1.475 ± 0.1	39.64 ± 0.8	0.159 ± 0.005	1.67 ± 0.2	3.3 ± 0.04	9.6 ± 0.12	6.8 ± 0.041	68.2 ± 2.11
AD sludge	17.25 ± 0.07	2.24 ± 0.06	1.71 ± 0.014	18.72 ± 0.08	0.78 ± 0.015	2.33 ± 0.01	6.5 ± 0.05	14.56 ± 0.31	0	18.94 ± 2.6



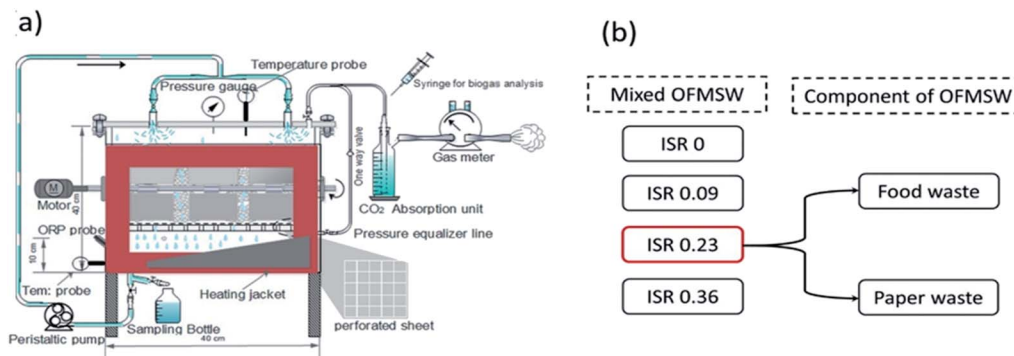


Fig. 1 (a) Schematic representation of percolator reactor and (b) experimental setup.

The reactor was manufactured by BAXH environment system Co. Ltd. Beijing. The reactor was designed, in accordance with the literature, with a surface to volume ratio of 2.5, to avoid side-effects at the reactor walls.³³ The height of reactor was divided into top and bottom sections and were separated by a perforated sheet (diameter 0.5 mm and 15% perforation area) positioned 10 cm from the bottom of the reactor. While this arrangement facilitates to hold the solid substrate, a geotextile sheet was placed on the perforated sheet to avoid migration of solid particles into a bottom section of the reactor, thus creating a 5 L volume at the lower part of the reactor for temporary leachate collection and storage. The leachate was recirculated with the help of a peristaltic pump (WT600-2J) to the upper part of the reactor and is uniformly distributed at the surface by means of a sprinkler. Backwashing, to clear the blockages in the perforated sheet, was done by using a peristaltic pump (in reverse direction).

Two sets of laboratory-scale experiments were performed to determine separation of biodegradable organic carbon from OFMSW at liquid to solid ratio (L/S) 5 (~48 g VS L⁻¹). Experimental setup is as shown in Fig. 1b. In the first set of experiments, three different ISR were used (0.09, 0.23 and 0.36) and compare with control (ISR 0) to determine the effect of ISR on hydrolysis and acidification for the optimization of process. In the second set of experiments, the acidogenic fermentation for sole components of OFMSW (PW and FW) at optimized ISR 0.23 to explore the synergistic effect.

All the experiments were done in duplicate, while the optimized batch was run in triplicate to ensure the authenticity of the results. The percolation reactor was operated with a hydraulic retention time (HRT) of 120 h at mesophilic temperature 35 ± 2.

2.3. Analytical methods

The concentrations of total solids (TS), volatile solids (VS), chemical oxygen demand (COD), nitrogen-ammonium (NH₄⁺-N), pH, total kjeldahl nitrogen (TKN) were identified according to Standard Methods.³⁴ Crude protein and lipids concentration were determined by TKN (multiplied by a factor of 6.25) and Soxhlet extraction method with petroleum ether used as organic solvent respectively. Biodegradability batch analyses was performed in accordance with the guidelines defined by

Angelidaki.³⁵ The elemental analysis (CHNOS) was performed with an elemental analyzer (Equipment CE 440; EAI USA). Dissolved organic carbon (DOC) was measured by using TOC analyzer (TOC-Vcph), Shimadzu, Kyoto, Japan.

Volatile fatty acid (VFA) were measured using a gas chromatograph (GC-2014; Shimadzu, Japan) equipped with a capillary column (Stabilwax-DA, 30 m × 0.32 mm × 0.25 μm; Restek, Bellefonte, PA, USA) and flame ionization detector. A detailed description of the methodology to analyze VFAs is described in the literature elsewhere.³² The concentration of proteins and reducing sugars were measured by using Lowry-Folin with bovine serum albumin (BSA) as standard,³⁶ Dinitrosalicylic acid method with glucose as a standard,³⁷ respectively. The samples were centrifuged for 10 minutes at 10 000 rpm and then were filtered (0.45 μm). To determine soluble COD, DOC, VFA, TAN, reducing sugar and protein, the same method as discussed above was adopted. The VFAs yield was calculated as the sum of the measured acetic, *n*-butyric, propionic, iso-valeric, *n*-valeric and iso-butyric acids. The conversion factors used for determination of the chemical oxygen demand (COD) of soluble organic materials were 1.07 for g COD g⁻¹ acetic acid, 1.5 for g COD g⁻¹ protein (assumed as (C₄H_{6.1}O_{1.2}N)_x), 2.04 for g COD g⁻¹ valeric acid, 1.51 for g COD g⁻¹ propionic acid, 1.82 for g COD g⁻¹ butyric acid and 1.07 g COD g⁻¹ reducing sugar (C₆H₁₂O₆).

2.4. Analytical techniques

2.4.1 Hydrolysis efficiency. The hydrolysis efficiency (η_{Hy}) is defined as the ratio of sCOD in the leachate to the initial dry chemical oxygen demand (TCOD) of the biodegradable substrate, is calculated by eqn (1).

$$\eta_{\text{hydrolysis (sCOD)}} = \frac{\text{sCOD}_{t=i}}{\text{TCOD}_{t=0}} \times 100 \quad (1)$$

2.4.2 Acidification efficiency. The acidification efficiency (η_{Ac}) is defined as the ratio of the cumulative total VFA (COD) and soluble COD in the leachate and is calculated from eqn (2)

$$\eta_{\text{acidification (sCOD)}} = \frac{\text{VFA}_{\text{sCOD}, t=i}}{\text{sCOD}_{t=i}} \times 100 \quad (2)$$

where TCO_D is the initial total COD (g) concentration of the substrate; sCOD is the cumulative soluble COD concentration



(g) with a particular time in the leachate, and VFA is the cumulative total VFA concentration (g COD) in the leachate.

2.4.3 Kinetics. Solubilization or liquidation of OFMSW is generally considered as two-step processes, hydrolysis, and acidogenesis; it can be expressed in eqn (3)



where OFMSW is a polymeric biodegradable substance of carbohydrate, protein, and fat (*i.e.*, volatile solids), it gradually hydrolyzed into soluble monomers (*i.e.*, sCOD) that can be further converted by acidogenesis into the fatty acids, *i.e.*, VFA (C₂–C₆). The hydrolysis step can be described by the first-order reaction kinetics, which has been considered as the most appropriate for complex heterogeneous substrates.^{19,38,39} According to Vavilin *et al.*³⁹

The relationship between substrate and product under the first order kinetics, it expressed in eqn (4) and (5).

$$\frac{dVS}{dt} = -kVS \quad (4)$$

$$\frac{dsCOD}{dt} = \alpha kVS \quad (5)$$

where VS is the volatile solids (VS) concentration, sCOD is the product concentration, *k* is the first-order rate constant, and α is the conversion coefficient of substrate to product. After integration of eqn (4) and (5), the product concentration is expressed as

$$sCOD = sCOD_{i=0} + \alpha VS_{i=0}(1 - e^{-k_{\text{h}}t}) \quad (6)$$

where sCOD₀ and VS₀ are the initial product and substrate concentrations, respectively. A non-linear regression would be used to estimate the values of hydrolysis rate reaction (per hour) and coefficient α and their standard deviations. The logistic

model is widely used for acidogenesis kinetics, which is related to the bacterial activity^{38,40,41} as shown in eqn (7)

$$\text{VFA}_{t=i} = \frac{\text{VFA}_{\text{mix}}}{1 + \left(\frac{\text{VFA}_{\text{mix}}}{\text{VFA}_{t=0}}\right)e^{-K_{\text{VFA}}t}} \quad (7)$$

where VFA, VFA_{mix}, and *K*_{VFA}, are the VFAs concentration at time *t* and the maximum VFAs concentration, apparent specific rate constant h⁻¹ respectively.

3. Results and discussion

3.1. Effect of ISR on process performance of acid fermentation

3.1.1 Bulk characterizations of leachate parameters: pH, soluble organics, NH₄⁺-N and alkalinity. The evolution of the main leachate parameters, such as pH, ammonia, alkalinity and soluble organic components, happened during the acid fermentation at different ISR (namely 0, 0.09, 0.23 and 0.36, respectively), are shown in Fig. 2.

The stable performance of acid fermentation is influenced mainly by the accumulation of organic acids and decrease of pH. The positive effects of ISR addition on pH increase at the initial stage of fermentation were observed. In particular, an initial pH value of 6.4–7.2, when using ISR > 0.09. As the fermentation proceeded, the pH values of reactors with ISR 0.23 and 0.36 decreased slowly and maintained a relatively stable level of 5.5 throughout the experimental period. However, pH values in ISR 0.09 and control reactors were dropped to ~4.0–4.5, which is below the optimal value for acid fermentation.¹²

The results regarding ammonia concentration are pointed out in Fig. 2a. Ammonia concentrations in ISR 0.23 and 0.36 reactors showed an increasing trend with time and reached to ~1100 mg L⁻¹ by the end of the experiment. In contrast, the

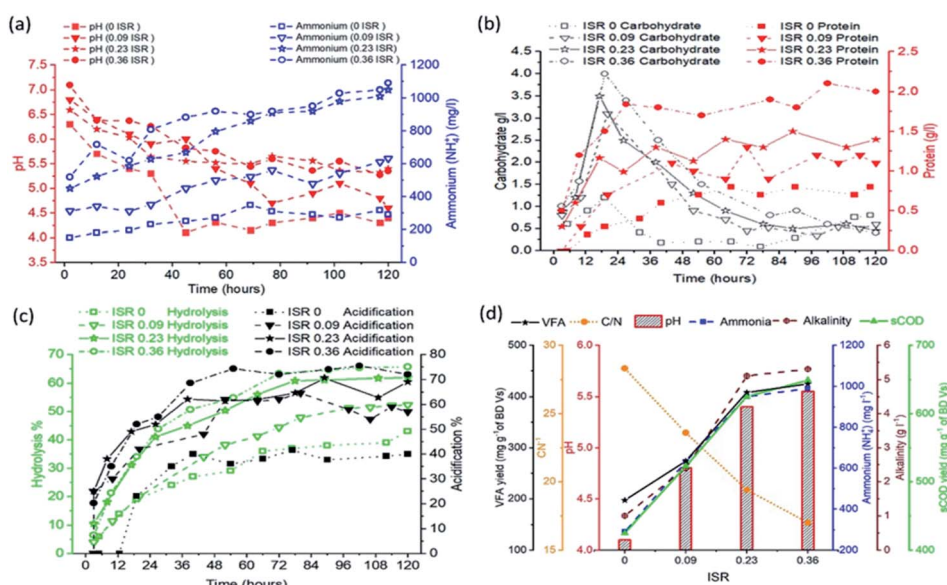


Fig. 2 Results of acid fermentation of OFMSW at different ISR (0–0.36) values (a) pH and ammonia, (b) soluble carbohydrate and protein released (c) hydrolysis and acidification efficiency (d) summary of overall results at the end of experiments.



initial $\text{NH}_4^+\text{-N}$ concentrations in ISR 0 and 0.09 reactors were obviously lower, and increased slightly in the subsequent days of acid fermentation, with the final values of 300 and 600 mg L^{-1} , respectively (Fig. 2d). These discrepancies of ammonia concentration among each reactor can be attributed to the higher protein content due to higher ISR (*e.g.*, more inoculum addition) (Table 2). Furthermore, the obtained ammonia could provide alkalinity (Fig. 2d) and neutralize VFAs produced in acid fermentation,²⁰ thus resulting in higher pH values in the fermentation liquor (~ 5.5).

The soluble carbohydrate concentrations in all the reactors decreased rapidly after the initial peak at hours 18–24 (Fig. 2b).

However, it was observed that the maximum concentration of soluble carbohydrate was 4000 mg L^{-1} at ISR 0.36 which was almost 4-folds higher than that without inoculum. This result can be explained by accelerated hydrolysis achieved due to the application of high ISR. In contrast, the concentration of soluble protein increased gradually in all the reactors, implying the biological transformation of protein usually occurs after carbohydrate degradation and requires extended period.⁴²

3.1.2 VFAs profiles. The evaluation of VFA composition is important as it provides useful information regarding the metabolic pathway and helps to understand its end-user

application. The evolution of VFAs production at different ISR during the whole experimental period is given in Fig. 3.

With the progress in hydrolysis and acidification process, the concentrations of VFAs increased steadily in all reactors, ranging from 16 to 24 g COD L^{-1} by the end of the study. It should be noted that the increase in ISR enhanced the VFAs production. At ISR 0, it was observed that the VFAs production was $\sim 197 \text{ mg g}^{-1}$ VS, which was the lowest yield in all ISR reactors.

The VFAs production from ISR 0.23 and 0.36 reactors reached 377 and 410 mg g^{-1} VS, respectively, which are ~ 1.9 and ~ 2.1 times higher compared with the control.

During the later phase of test, the concentrations of VFAs from ISR 0.23 and 0.36 reactors still maintained at relative and high level, possibly as the accumulation of fatty acids alters the system buffering condition leading to retardation of the rate of VFA production.

Butyric was dominating in all batches during the initial stage. Subsequently, butyrate-type fermentation was observed corresponding to the low ISR (*e.g.* 0 and 0.09), possibly due to pH effort. In contrast, it was observed that when $\text{ISR} \geq 0.23$, butyrate type fermentation shifted to mixed-type and acetic-type fermentation which includes 42–53% of acidic acids and $\sim 14\%$ of propionic. Hawkes *et al.*⁴³ reported that, with the increase in

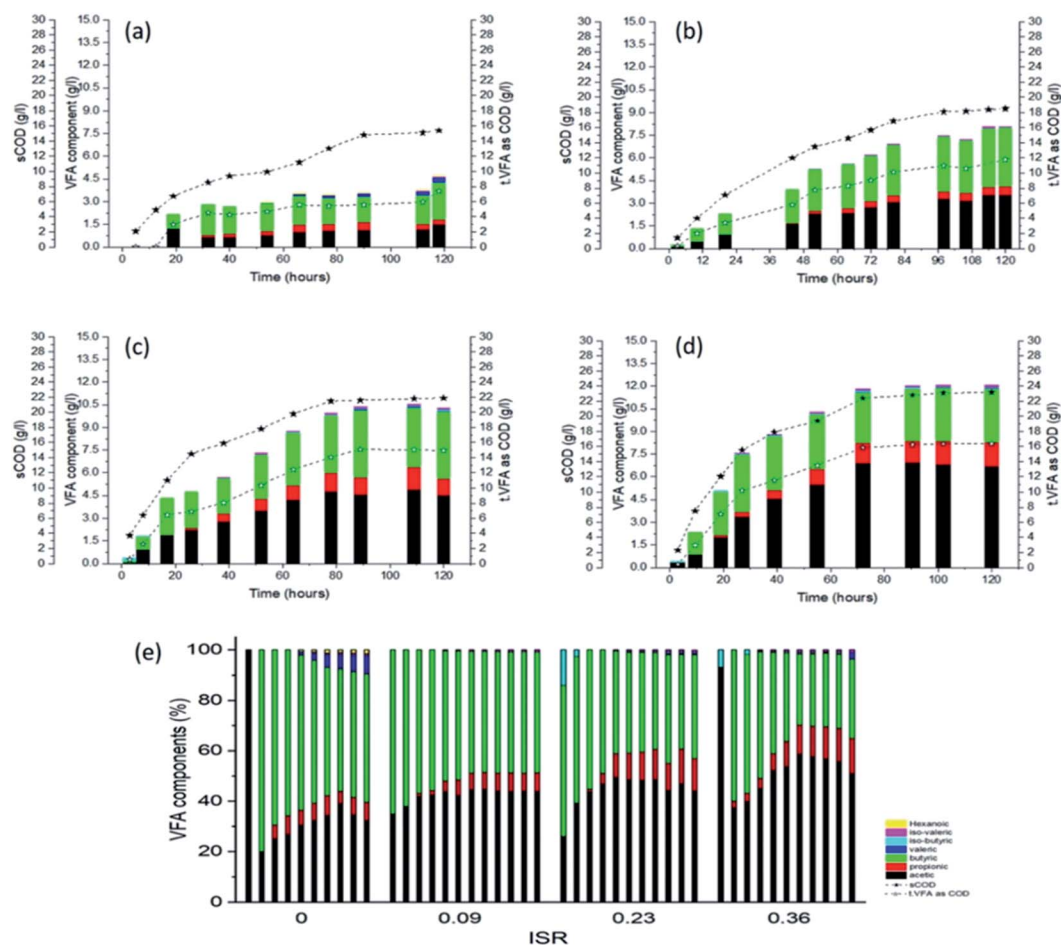


Fig. 3 sCOD and VFA concentration and VFA composition under different ISR from OFMSW. (a) ISR 0, (b) ISR 0.09 (c) ISR 0.23 (d) ISR 0.36 and (e) overall compression of VFA compositions.



the pH, butyric was converted to acidic and propionic acid and the authors attributed the change could be because of change in the dominant microbial species or change in the metabolic pathway within the same bacterial population. In the present study, the propionic acid accumulation was noticed at relatively low levels in ISR 0.23 and 0.36 reactors, which was suitable for the subsequent usage, such as methane production.

The comprehensive comparison of VFAs production in this study with the studies reported in literature is showed in Table 4. The positive effects of optimizing ISR addition, without pH adjustment, on acids production were satisfactory.

3.1.3 Hydrolysis and acidification extent. The hydrolysis and acidification efficiencies were calculated using eqn (1) and (2), respectively, and are shown in Fig. 2c. Additionally, the kinetic parameters were also analysed according to the eqn (3), by applying first-order kinetics eqn (6) and logistic model eqn (7), and the result are depicted in Table 3 and Fig. 4.

The increase in ISR had a direct influence on the hydrolysis and acidification efficiencies: the highest hydrolysis and acidification efficiencies were 64% and 74%, respectively, at ISR 0.36, followed by ISR 0.23 (η_{Hy} : 62 and η_{Ac} : 70), ISR 0.09 (η_{Hy} : 50 and η_{Ac} : 60) and control ISR 0 (η_{Hy} : 40 and η_{Ac} : 40). Overall, hydrolysis rate as stipulated by the increasing production in sCOD from 0.019 to 0.038 per hour was observed as ISR was increased from 0 to 0.36. ISR 0.036 had a maximum value for α , K_h and VFA_{max} , which were 0.456, 0.0381 h^{-1} and 15.9 g L^{-1} , respectively. These values were almost two-fold higher than the control (ISR 0). The hydrolysis rate constant (0.91 per day)

obtained at the ISR 0.36 in this study was much higher than the reported value,⁴⁴ which was due to high OLR used in this study.

However, it is worth to note that a slightly decrease of K_{VFA} appeared with ISR 0.36 as compared to ISR 0.23, which might be attributed to quicker VFAs degradation with more inoculum addition. Similar result was also reported by Tomei *et al.*⁴⁵ Hence, ISR 0.23 was chosen as optimal parameter in order to avoid the undesired loss of organic carbon in substrate.

3.2. Comparison of individual component and OFMSW for the process performance

The pH of components of OFMSW (PW and FW) and mixed OFMSW were not adjusted artificially during fermentation process; they fluctuated in a range of 7.2–7.5, 4.1–6.5 and 5.4–6.5, respectively. Sole PW and FW fermented at ISR 0.23 and 120 HRT is shown in Fig. 5.

The soluble products during the acidogenic fermentation of sole PW was observed to be lowest and, in contrast, the pH was observed to be highest as compared to FW and OFMSW. Due to the high C/N ratio, around 96, which imply that sufficient nitrogen is not available for the synthesis of bacteria to degrade the cellulosic natured substrate. This could be the possible reason for not recommending sole PW for acidification.³⁰

Sole FW hydrolysis was noticed to rapidly increase up to 66% with highest production of sCOD 28 g L^{-1} . On the other hand, pH rapidly dropped to reach the lowest value, 4.2, during the first 60 h. Acidification efficiency started from 40%, which was highest.

Table 3 Kinetic parameter for the hydrolysis and acidification steps during OFMSW fermentation at different ISR

Stage	Parameters	ISR 0	ISR 0.09	ISR 0.23	ISR 0.36
Hydrolysis	α	0.307 ± 0.012	0.371 ± 0.023	0.4345 ± 0.041	0.456 ± 0.047
	K_h (h^{-1})	0.019 ± 0.005	0.022 ± 0.0063	0.0366 ± 0.0071	0.0381 ± 0.0052
	R^2	0.976	0.998	0.992	0.997
	SE	0.003	0.0012	0.004	0.006
	VFA_{max} (g L^{-1})	7.395 ± 0.347	11.722 ± 0.417	13.53 ± 0.340	15.9 ± 0.573
Acidogenesis	K_{VFA} (h^{-1})	0.0465 ± 0.0049	0.0494 ± 0.0057	0.0803 ± 0.0099	0.0790 ± 0.0113
	R^2	0.965	0.978	0.979	0.962
	SE	0.0012	0.002	0.008	0.0009

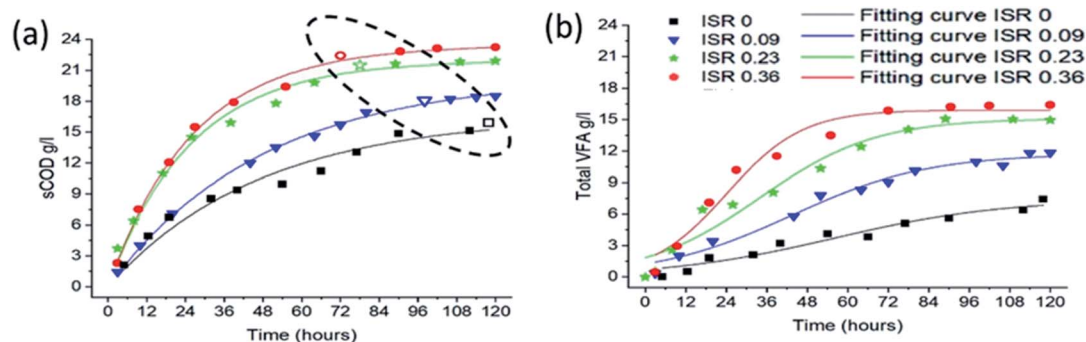


Fig. 4 Kinetic analysis of different ISR during the hydrolysis and acidification process. (a) sCOD production under different ISR. (b) T VFA production under different ISR.



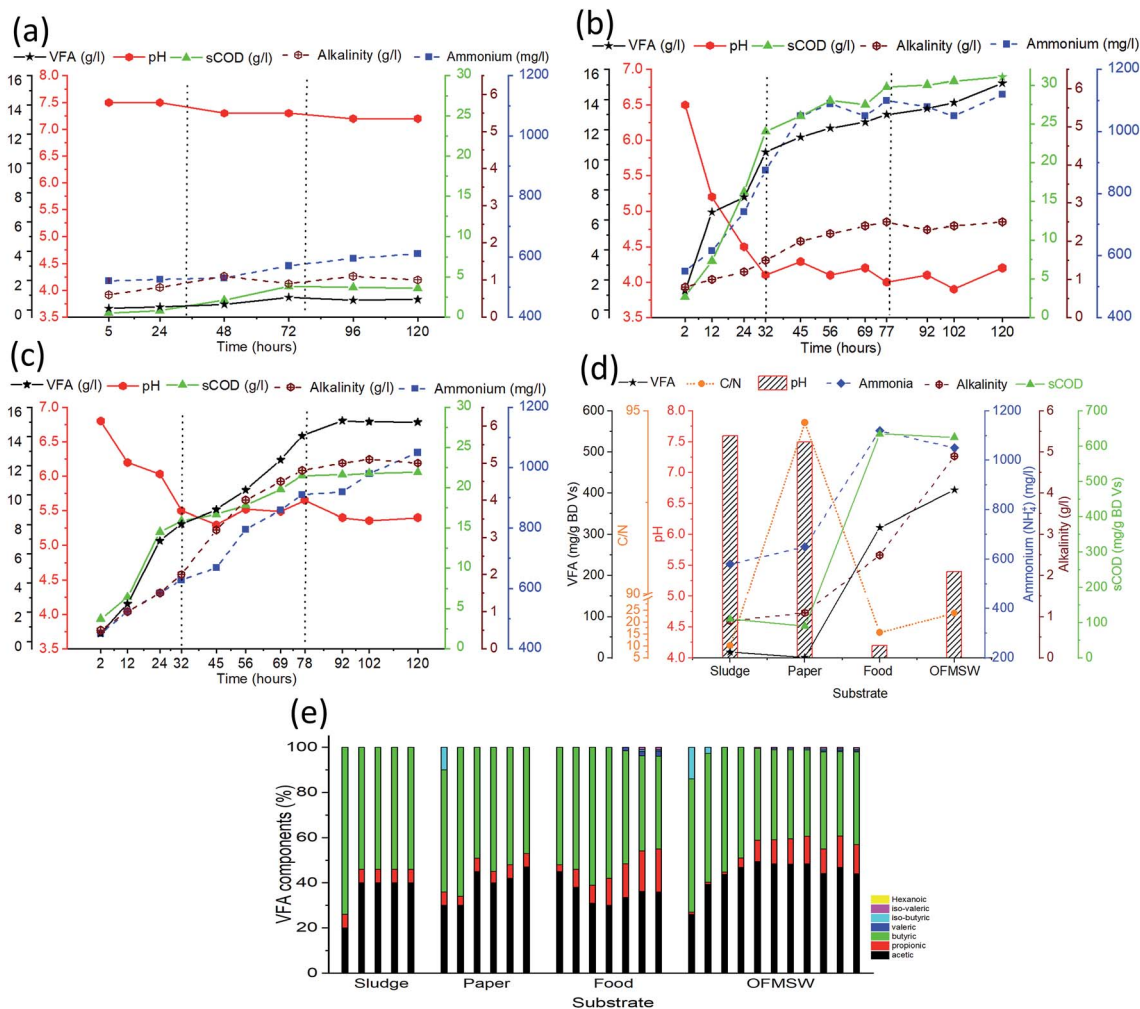


Fig. 5 sCOD and VFA production of mono and mixed fermentation at ISR 0.23. (a) Mono fermentation of PW (b) mono fermentation of FW (c) mixed fermentation of OFMSW (d) summary of overall result and (e) overall composition of VFA from mono to mixed fermentation.

However, acidification efficiency was almost same throughout the experiments while the efficiency of OFMSW increased up to 70% at the end of experiments. It was noticed that the accumulation of carbohydrate and protein was 2 and 2.5 g L⁻¹, respectively, which is *ca.* two-fold higher than the OFMSW. These results indicated that process of acidification and hydrolysis was suppressed due to the low pH 4.2, the organic acids freely pass through the cell membrane of microbes, consequently inhibited the overall acidogenic process.⁴⁷ Similar results were reported by Jiang *et al.*⁵ and Wu *et al.*^{6,21}

Acidogenic fermentation of mixed OFMSW (FW and PW at mixing ratio of 55 : 45 by VS), during the initial phase of hydrolysis, soluble part of carbohydrate and protein from FW rapidly converted into VFA which caused a sudden drop in pH to 5.4 within 32 hours, which is 26% higher than sole FW. Consequently, the hydrolysis efficiency and acidification efficiency reached to 45% and 40%, respectively. Ammonia concentration showed contrasting values, *i.e.*, 1050 mg L⁻¹,

8.6% lower than FW and plausible reason could be the higher protein content in FW as compared to OFMSW as shown in Fig. 5 and Table 2. It can be concluded that ammonia is not the only parameter responsible for the pH stabilization in mixed fermentation.

After initial phase, PW would be hydrolyzed due to favorable pH conditions.⁴⁸ Hydrolysis of PW provides two opportunities, (1) cellulose degrade into glucose, which is the most suitable substrates for VFA production. (2) It releases carbonates, a major additive in PW which accounts *ca.* 21%. Ultimately, in this case, alkalinity increased from 2 to 5 g L⁻¹ which is two-fold higher than sole FW. In this way performance of mixed fermentation process was much better than the sole FW, especially after the initial phase^{31,49} shown in Fig. 5a, the pH of mixed fermentation was in the range of 5.3–5.5, is optimal for hydrolysis as well as acidification.²¹ The hydrolysis and acidification efficiency, and ammonia gradually increased from 40–64%, 50–70%, and 600–1050 mg L⁻¹ respectively. The soluble carbohydrate and protein were stable due to their balanced



conversion to VAF. During the course of time the overall pH was stable. The results demonstrated that fermentation of OFMSW concurrently accelerated the substrate acidification. It affects the composition of VFA and butyric acid was reduced from 60–40% in contrast to the acetic acid which increased from 26–41%, due to the pH uplift from 4.2 to 5.4 (sole FW to mixed OFMSW) recorded among all batches investigated in the present study and it might be due to plenty of soluble carbohydrate available in fermentation broth.²¹

3.2.1 Synergistic effect. The synergistic effect is the internal reaction produced by the fermentation of different types of substrate.⁵⁰ In current study, two individual components from OFMSW (paper, and food) compared with mixed OFMSW were selected. Moreover, it can be mathematically expressed in eqn (8).

$$\alpha = \text{Experimental Yield/Theoretical Yield} \quad (8)$$

where “Experimental Yield” represented as a hydrolysis and acidification yield obtained from fermentation of OFMSW. “Theoretical Yield” was calculated as the sum of soluble products, each substrate multiplied by its percentage in the mixture of biodegradable portion of VS. “Theoretical Yield” can be calculated as eqn (9).⁵¹

$$\text{Theoretical yield} = \text{yield}_{\text{Paper}} \times \text{paper}_{\%BD} + \text{yield}_{\text{Food}} \times \text{food}_{\%BD} \quad (9)$$

where, $\text{yield}_{\text{Paper}}$ and $\text{yield}_{\text{Food}}$ are the experimental sCOD and VFA yields of mono-fermentation of PW and FW respectively. $\text{Paper}_{\%BD}$ and $\text{food}_{\%BD}$ are referred to the mixing ratio from fermentation mixture. Synergistic effect evaluation was based on the biodegradables, COD and carbon. Summarized synergistic analysis for mixed fermentation of PW and FW is presented in Table 5. Zero effect consider being for inoculum on sole and mixed in acidogenic fermentation. Because sludge has

low biodegradation around 15% and its biodegradable part contributing to VFA and soluble carbohydrate is less than 10%, it showed very negligible effect.

Overall maximum synergistic effect was found on acidification (VFA) with 76% and 59% of carbon and COD, respectively. It also affected hydrolysis in 35% and 24.6% of carbon and COD respectively. A slight synergistic effect has been observed in biodegradability. There are two plausible possible reasons for synergies enhancing during hydrolysis and acidification; the first reason could be due to achieved pH (5.2–5.6) which is favorable for acidogenic fermentation (detail discuss in pH section).¹² Same results were observed during the co-fermentation of food and excess sludge.²¹ At this pH, PW hydrolyzes into soluble carbohydrate; it was the preferred substrate by the microbes. It is firstly degraded to glucose, then to pyruvate. Pyruvate is an important intermediate product that is readily oxidized to acetyl-CoA, which can be further utilized to produce acetic and butyric by diverse enzymatic actions.⁵² Elliston *et al.*⁵³ reported that the PW would be hydrolyzed under pH 5–5.5. The second reason might be microbial shift change due to the mixed fermentation, although no significant evidence was observed in the present study. It was suggested that bacteria, such as *Clostridium* the ocellus, may facilitate the extraction of cellulose from waste paper, which facilitates the degradation of cellulosic materials.⁵⁴ Further research is required to determine the presence of cellulose-secreting microorganisms within the culture.

3.3. Carbon balance analysis

The carbon mass balance explains the electron flow through the anaerobic process¹² and optimized conditions (ISR 0.23, results discussed in Section 3.2) were applied for PW, FW and OFMSW and shown in Fig. 6. In general, carbon represents the organic carbon from organic waste in the form of biodegradable portion of VS. It is reported that the organic carbon can be converted

Table 4 Comparison of VFAs production from different modes of fermentation and at different operational conditions in literature^a

Substrate	Seeding	Optimal condition		Reactor mode and capacity	Ac: % & Hy: %	VFA production	pH	Ref.
		Temp* °C, OLR ⁺ g VS L ⁻¹ , ISR [^] , SRT ^x days & particle size [†] mm						
FW : ES	WAS	40*, 9 ⁺ , 0.2 [^] , 7 ^x & ND [†]		Semi-conti.	Ac: 83% & Hy: 63%	867 mg g ⁻¹ VS	5.2 not fixed	21
FW	AD sludge	35*, 11 ⁺ , 8 ^x & ND [†]		Batch, 4.5 L	Ac: 75%	471 mg g ⁻¹ VS	6 adj	6
FW	AD sludge	35*, 10 ⁺ , 0.5 [^] 10 ^x		Batch, 0.5 L	Ac: 80% & Hy: 31%	ND	5.5 adj	11
OFMSW ^a	AD sludge	55*, 44.4 ⁺ , 1.9 ^x & 15 mm		Semi-conti., 10 L	Ac: 61% & Hy: 59.6%	ND	5–5.5 adj	46
FW	AD sludge	35*, 9 ⁺ , 8 ^x & ND [†]		Semi-conti., 2 L	Ac: 63%	333 mg COD	5.5 adj	28
OFMSW ^b	A.S.C	35*, 28 ⁺ , 80 ^x & 4 [†]		LBR, 5 L	Ac: 60% & Hy: 61%	7 g L ⁻¹	5.5 adj	26
OFMSW	AD sludge	37*, 48 ⁺ , 0.23 [^] 3.25 ^x & 40–50 mm		Percolator B, 20 L	Ac: 65% & Hy: 64%	377.5 mg sCOD	5.5 not fixed	Current study

^a A.S.C = acidogenic seed culture. ND = not define (crushed by an electrical blender)[†], FW = food waste, KW = kitchen waste, ES = excess sludge, AD = anaerobic digestion. WAS = waste activated sludge, PW = paper waste, Ac = acidification, C. Ac = carbon acidification, Hy = hydrolysis, SRT = solid retention time Adj = adjustable. OFMSW^a derived from MBT and component doesn't show. OFMSW^b derived from mixture of fruit, vegetable and kitchen waste.



Table 5 Synergistic effects of hydrolysis and acidification during fermentation of OFMSW

Parameters		Paper	Food	OFMSW (theoretical)	OFMSW (experimental)	Synergistic effect
COD	Biodegradability	0.6	0.7	0.655	0.7	1.069
	sCOD ($\text{g}^{-1} \text{L}$)	3.5	28.5	17.25	21.5	1.246
	VFA ($\text{g}^{-1} \text{L}$)	0.805	15.105	8.813	14.1	1.59
Carbon	Biodegradability	0.55	0.65	0.605	0.65	1.074
	DOC ($\text{g}^{-1} \text{L}$)	1.56	9.8	6.092	8.2	1.35

into a wide range of products during hydrolysis–acidification process of anaerobic digestion.

Mainly it can be converted into three forms, (1) soluble liquid fraction, into VFAs, proteins, carbohydrate, lactic acid, and alcohols.⁶ (2) Gaseous fraction, carbon dioxide and methane,¹² (3) solid fraction (solid residue).

The gaseous and solid fraction were measured at the start and end of the experiment, while the soluble liquid fraction was measured during the experiment. Gaseous fraction and suspended part of the liquid fraction was mathematically calculated by subtracting the soluble liquid and final solid residue from the initial carbon that is represented as “uncountable carbon.” The quantity of carbon in a soluble liquid fraction was quantified in terms of DOC. Furthermore, carbon distribution in a soluble protein, carbohydrate and VFA were measured according to the molecular weight of the chemical formula.¹⁵

Sole sludge effect was negligible because sludge has a low biodegradability around 15% as shown in Table 5. A major portion of carbon in sludge was accountable for solid residue and uncountable part of carbon. The carbon represented by solid residue and VFA were dominant in paper, food and OFMSW, respectively. Additionally, the solid residue mass in PW, protein, carbohydrate and uncountable mass in FW also showed greater percentages than in OFMSW and were 80%, 12%, 6% and 10% of the total carbon, respectively.

This data indicated that the fermentation substrates in PW and FW were not adequately utilized to produce VFAs. However, the carbon masses of protein, carbohydrate and uncountable carbon in FW are much higher than OFMSW, these parts of carbon might be converted into VFA and another soluble part of the carbon into OFMSW.

Biodegradable carbon represented by VFA in FW and OFMSW were 30% and 42%, respectively. Mixed fermentation

has more than 70% of biodegradable solid carbon converted into liquid carbon, which is more than the sole component of OFMSW (FW and PW).

4. Conclusions

A novel, practical and cost-effective strategy for the converting biodegradable carbon of OFMSW into VFA *via* acidogenic fermentation without pH control has been proposed. The optimized VFA production by fermentation of OFMSW (FW + PW) at ISR 0.23 adjustment was attributed to favorable pH, 5.5–5.2, achieved, which subsequently enhanced the hydrolysis and acidification. The maximum synergies were observed during the acidification stage of fermentation, 1.76-fold greater as compared to individual substrate. Hydrolysis and acidification yields were observed to be 625 mg sCOD per g BD VS and 408 mg g^{-1} BD VS respectively. More than 80% of BD carbon was recovered from OFMSW within 78 h, out of which more than 70% was solubilized into leachate.

An approximate 55% of dissolved biodegradable organic carbon was available in the form of VFA and major composition was acetic acid and butyric acid followed by propionic acid, which could be considered as the suitable substrate for methane production *via* acetotrophic methanogenesis metabolic pathway. Furthermore, the leachate contains soluble part of a protein, carbohydrate and the suspended carbon, which might be further hydrolyzed during methanogenesis to enhance methane production. Kinetics of hydrolysis and acidification rate was calculated and the k_h values were noted to be 0.036 h^{-1} and 0.08 h^{-1} , respectively.

Conflicts of interest

There are no conflicts to declare.

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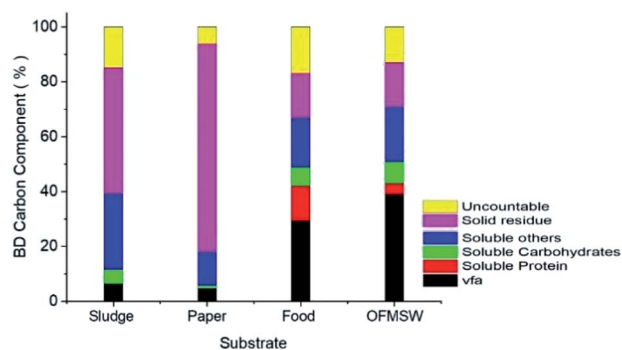


Fig. 6 Percentage of different component of biodegradable carbon of mono and mixed fermentation, at HRT 120 h.



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