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Effects of operational factors on soluble microbial products in a carrier anaerobic baffled reactor treating dilute wastewater

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Abstract

The effects of feed strength, hydraulic residence time (HRT), and operational temperatures on soluble microbial product (SMP) production were investigated, to gain insights into the production mechanism. A carrier anaerobic baffled reactor (CABR) treating dilute wastewater was operated under a wide range of operational conditions, namely, feed strengths of 300–600 mg/L, HRTs of 9–18 h, and temperatures of 10–28°C. Generally, SMP production increased with increasing feed strength and decreasing temperature. At high temperature (28°C), SMP production increased with decreasing HRT. As the temperature was decreased to 18 and 10°C, the SMP production was at its peak for 12 h HRT. Therefore, temperature could be an important determinant of SMP production along with HRT. A higher SMP to soluble chemical oxygen demand (SCOD) ratio was found at high temperature and long HRT because of complete volatile fatty acid degradation. SMP accounted for 50%–75% of the SCOD in the last chamber of the CABR. As a secondary metabolite, some SMP could be consumed at lower feed strength.

Key words: carrier anaerobic baffled reactor; dilute wastewater; soluble microbial product; hydraulic residence time; feed strength; temperature

Introduction

On account of stringent effluent standards, a deeper understanding of the material leaving a biological waste treatment system is required. Soluble microbial products (SMPs) are always formed during a treatment and their presence in the effluent, even in well-established systems, may reflect their refractory nature (Aquino and Stuckey, 2004).

The formation of SMPs influences the treatment process in many ways. SMP formation is a matter of great interest, not only in terms of achieving discharge standards, but also in setting the lower limit of treatment. Therefore, determining the factors that affect SMP production is of paramount importance (Aquino and Stuckey, 2003). At low substrate levels, SMPs are particularly important in determining the effluent quality (Barker *et al*., 2000).

On account of difficulties in measuring SMP quantities, a comprehensive definition of SMP is somewhat ambiguous and depends on what viewpoint is considered. From an engineering point of view, the most widely accepted definition is that it is a pool of organic compounds that results from substrate metabolism (usually with biomass growth) and biomass decay (Barker and Stuckey, 1999). For aerobic systems this is usually quite clear; however, for anaerobic systems the intermediate short-chain volatile fatty acids (VFA) are excluded from SMP. A basic operational definition is any soluble material that appears in the effluent and was not present in the influent (Barker and Stuckey, 1999).

The generation of SMP is typically divided into two categories: biomass-associated products (BAP) (SMP associated with biomass decay) and utilization-associated products (UAP) (SMP associated with substrate metabolism and, typically, biomass growth) (Noguera *et al*., 1994; Yu *et al*., 2006). The UAP are produced at a rate proportional to the rate of substrate utilization and BAP are produced at a rate proportional to the concentration of the biomass. Modeling studies carried out on SMP production in anaerobic systems, using radiolabelled compounds, suggest that UAP are more biodegradable than BAP (Barker and Stuckey, 2001).

These SMPs may not be readily biodegradable, or may even be refractory, and comprise of a wide range of organics distributed across a broad spectrum of molecular weights (Aquino and Stuckey, 2003; Boero *et al*., 1996), such as, humic acid, fulvic acid, polysaccharides, proteins,

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nucleic acids, organic acids, antibiotics, steroids, enzymes, and structural components of cells (Barker *et al*., 2000).

Some workers have shown that the normalized production of SMP is less in anaerobic systems (0.2%–2.5%) than in aerobic systems (3.1%–14.7%), for well-operated systems (Boero *et al*., 1991), and it has been demonstrated that more SMPs are produced during acidogenesis than during methanogenesis (Barker and Stuckey, 1999).

SMP production is directly proportional to the feed strength, and different amounts of SMP are produced when different simple substrates are fed into the system (Barker and Stuckey, 2001). It is also believed that SMP may be produced in response to environmental stress, such as, extreme temperature changes (Smeaton and Elliot, 1967), osmotic shock (Rogers, 1968), nutrient deficiency (Aquino and Stuckey, 2003), and the presence of toxic compounds (Kuo and Parkin, 1996). It has been observed that pure cultures of bacteria excrete organic compounds to establish concentration equilibrium across the cellular membrane (Payne, 1976). In the 1970s, many studies have been carried out using pure cultures, which suggest that microorganisms can produce microbial compounds as a result of starvation and an excess energy source (Boylen and Ensign, 1970; Neijssel and Tempest, 1976). It is not surprising that the most important factors affecting SMP production during normal growth and metabolism are hydraulic residence time (HRT) and SRT (sludge retention time) (Aquino and Stuckey, 2004).

Although the significance of SMP formation has been emphasized in the literature, limited information is available on the effect of short shock loads on SMP production in an anaerobic reactor (Liu *et al*., 2002; Fang *et al*., 2006), especially for a carrier anaerobic baffled reactor (CABR), treating dilute wastewater.

The CABR has a packing made of hollow-sphere bamboo, with a high specific surface area (2,100 m^2/m^3), which was developed by Shen (2006), at the Zhejiang University. It combines the advantages of an anaerobic baffled reactor and the characteristics of a biofilm reactor. It is a new high-rate anaerobic reactor for decentralized treatment, which has shown good performance in the removal of organic pollution and entrapping suspended solids (SS).

On the basis of the authors' previous studies and other literature (Grobicki and Stuckey, 1992), the hydraulic characteristics of CABR are intermediate between those of a plug flow reactor and continually stirred tank reactor, and about 95% of the original water can be replaced after about twice the HRT. Therefore, using twice the HRT as the time duration is feasible for a short-shock load study. Moreover, domestication of microorganisms still occurs to some extent.

The present investigation studies the effects of feed strength, hydraulic residence time, and operational temperatures on SMP production, using a CABR to treat dilute wastewater. In addition, the authors attempt to explore the mechanism of SMP formation.

1 Materials and methods

1.1 Experimental setup

The CABR was made of 8 mm thick transparent Perspex (Fig.1), 600 mm in length, 140 mm in width, and 300 mm in depth. The reactor was rectangular, containing six chambers of equal volume with an active volume of 17 L. The width of each chamber's down-comer was 25 mm, whereas, each up-comer was 60 mm. The liquid sampling ports were located 200 mm behind the effluent port of each chamber. The six up-comer regions were filled with hollow-sphere carriers made of bamboo (diameter of 1.5 cm). The carriers had a high specific surface area, which reached 2,100 m^2/m^3 with a high porosity of 95%. The bamboo carriers enabled biomass retention through attaching and were able to entrap SS from the domestic sewage.

1.2 Seed sludge

The CABR was seeded with screened digested sludge (about 3.5 L) from the Sibao Wastewater Treatment Plant Hangzhou in Zhejiang Province, China. The total suspended solids (TSS) and volatile suspended solids (VSS) of sludge were 23.8 and 9.2 g/L, respectively.

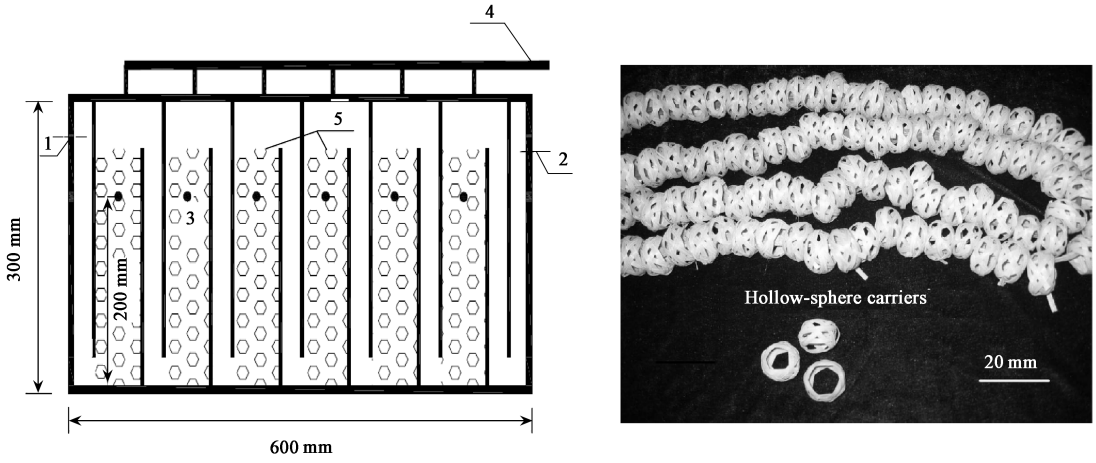

Fig. 1 Schematic diagram of the carrier anaerobic baffled reactor (CABR) with packing made of hollow-sphere bamboo. (1) influent inlet; (2) effluent outlet; (3) liquid sampling port; (4) biogas; (5) hollow-sphere carriers.

1.3 CABR startup

The CABR was initially fed with domestic sewage from Zhejiang University, mainly from restaurants and dormitories, for three months during startup, at 28 ± 1°C. The HRT was gradually decreased from 48 to 18 h by increasing the flow rate. The SCOD (soluble chemical oxygen demand) and TCOD (total chemical oxygen demand) removal efficiencies were 74% and 69%, respectively, at an HRT of 18 h.

1.4 Feed composition

To avoid problems of varying composition and degradability of the feed, a buffered synthetic carbohydrate-protein substrate supplemented with nutrients and trace metals was used, as shown in Table 1. The influent feed was pumped using a peristaltic pump.

1.5 Test procedures

Initially, the reactor was operated for one month with a feed strength of 300 mg/L chemical oxygen demand (COD), at an HRT of 18 h, and temperature of 28 ± 1°C, to provide a steady baseline for which the TCOD removal efficiency was (85.5 ± 2.4)%. A steady state was deemed to exist when the variation in the TCOD removal efficiency was less than 5% for three successive days. After establishing a steady state, the feed concentration was elevated to 450 mg/L COD at constant HRT, and the reactor was operated for 36 h (twice the HRT). The samples were collected before changing the operational conditions. The feed concentration was then increased to 600 mg/L COD and the operation that followed was similar to that described earlier. Subsequently, the operational parameters were adjusted to the baseline and used for 10 d with a feed strength of 300 mg/L COD at an HRT of 18 h. After reaching a steady state, the HRT was reduced to 12 h at 300 mg/L COD. Before changing the operational conditions, the reactor was operated for 24 h (twice the HRT) and a sample was collected. The HRT was then decreased to 9 h at the same feed strength. The reactor was operated for 18 h (twice the HRT) and samples were collected. The process temperature was controlled at 28 ± 1°C.

Next, the operational temperature was decreased to 18 ± 1°C by 1°C per day (Ndon and Dague, 1997). The reactor was operated for 10 d with a feed strength of 300 mg/L COD keeping an HRT of 18 h at 18 ± 1°C, to establish a steady baseline. After reaching a steady state, the subsequent operation was similar to the previous step except for the operational temperature.

Finally, the operational temperature was once again further decreased to 10 ± 1°C by 1°C per day. The third step was similar to the second step except for the temperature.

Table 1 Feed composition used in the study

Feed component	Weight (g)	Trace nutrients	Weight (g)
NH_4Cl	40	$CoCl_2{\cdot}6H_2O$	0.0238
Sugar	48	$FeCl_2{\cdot}4H_2O$	0.157
Urea	1.8	$MnCl_2{\cdot}4H_2O$	0.0075
K_2HPO_4	8.4	$Na_2MoO_4{\cdot}2H_2O$	0.0075
KH_2PO_4	6.6	$NiCl_2{\cdot}6H_2O$	0.009
$NaHCO_3$	60		
Peptone	60		
Sodium dodecyl-benzenesulfonate	0.8		
Humic acid	0.8		

Constituents were used to prepare 10 L of feed with a chemical oxygen demand (COD) concentration of 12 g/L.

1.6 Analytical methods

All the analytical methods adopted for measurements of alkalinity, SS, COD, and pH were according to the standard methods (APHA, 1995). Raw samples were used for measuring the TCOD, whereas, 0.45-μm membrane-filtered samples were employed for SCOD determination.

Glucose was determined by using a HPLC system (Shimadzu, Japan), with a Polyspher OH HY column (Merck, Germany). The sample volume was 100 μl, the column was maintained at 35°C, and the effluent was 0.005 mol/L H_2SO_4 at a flow rate of 0.5 ml/min (Aquino and Stuckey, 2003). VFA were determined by gas chromatography. The chromatograph (Agilent 6890N, USA) was equipped with a 2 m × 2 mm (inner diameter) glass column, packed with Porapak GDX-103 (80–100 mesh). Operating temperatures were 140°C for the column, 240°C for the injection port, and 210°C for the flame ionization detector, and N_2 was used as the carrier gas (40 ml/min). All the samples for analysis of the VFA were first acidified with concentrated H_2SO_4 to a pH of approximately 3.5, and then centrifuged at 8,000 r/min, for 20 min. Finally 0.15 ml formic acid was added for each 3 ml sample and adjusted to pH < 2 (Ren *et al*., 2005).

One of the greatest difficulties for researchers was the actual measurement of SMPs (Yu *et al*., 2006). Following the previous literature (Barker and Stuckey, 1999; Kuo *et al*., 1996), SMP was defined as:

$$\mathrm{SMP} = \mathrm{SCOD} - 1.07(\mathrm{HAc}) - 1.51(\mathrm{HPr}) - 1.82(\mathrm{Hbu}) - 1.07(\mathrm{Glu})$$

where, HAc (mg/L) is the measured acetic acid, HPr (mg/L) is the measured propionic acid, Hbu (mg/L) represents the measured *iso*- and *n*-butyric acids, and Glu (mg/L) is the measured glucose. The values 1.07, 1.51, and 1.82 are conversion factors assuming the complete oxidation of volatile acids or glucose to CO_2 and H_2O. This definition of SMP has also been adopted in several other studies for anaerobic systems (Nachaiyasit and Stuckey, 1997a; Schiener *et al*., 1998).

1.7 Statistical analysis

Analysis of variance was performed using SPSS^{TM} v.13. A paired samples test was also performed.

2 Results and discussion

2.1 Effect of feed strength

As illustrated in Fig.2, the peak in SMP production was in the first chamber of the CABR. The results must be

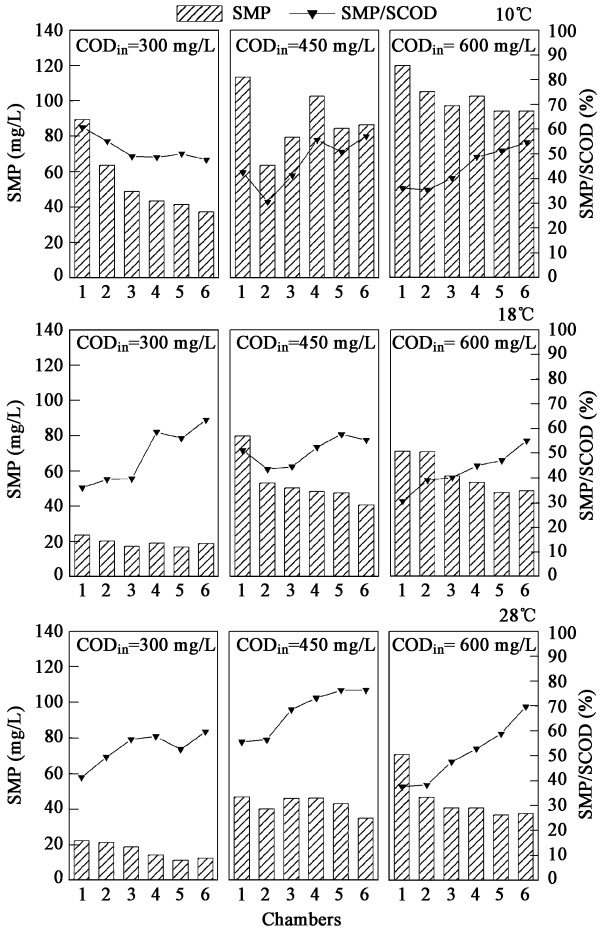

Fig. 2 Soluble microbial product (SMP) profile in the chambers at different temperatures and feed concentrations. SCOD: soluble chemical oxygen demand.

interpreted with a degree of caution because the nondegraded substrate could not be measured (Barker *et al*., 2000), and therefore, the calculated SMP concentrations also included any residual substrate. The error was greater at low temperatures and high feed strengths because of the slower rate and less time available for biodegradation. To determine the exact SMP concentration, the mean SMP concentration in the last three chambers was considered as the mean value for the entire reactor. Table 2 shows that the SMP concentrations were 12.61 ± 1.49 mg/L, 41.37 ± 5.89 mg/L, and 38.32 ± 2.18 mg/L for the feed strengths of 300, 450, and 600 mg/L COD, respectively, at 28°C. A drastic increase in SMP concentration was observed when the feed strength was increased from 300 to 450 mg/L COD, whereas, there was no significant increase ($P > 0.05$) when the feed strength was further increased to 600 mg/L COD. By and large, the SMP concentration increased with decreasing temperature, and similar profiles were observed at 18 and 10°C, which agreed with the former studies (Nachaiyasit and Stuckey, 1997b).

The microorganisms were extremely deficient in nutrients at a feed strength of 300 mg/L COD and the SMP was partially consumed. SMPs were always shown to be biodegradable (both aerobically and anaerobically), although the kinetics of degradation could be slower than

Table 2 SMP for the entire reactor at different temperatures and feed strengths (n = 3, mean ± SD, mg/L)

Influent COD (mg/L)	Temperature			Average
	10°C	18°C	28°C	
300	40.57±3.18	18.22±1.30	12.61±1.49	23.80±14.79
450	91.03±9.89	45.54±4.15	41.37±5.89	59.31±27.55
600	96.99±4.80	49.94±3.09	38.32±2.18	61.75±31.07
Average	76.20±25.31	37.90±14.03	30.77±12.90	

for simple substrates (Barker *et al*., 2000; Liu *et al*., 2002). SMP, as a secondary metabolite, was not used when the feed was no longer a restriction for metabolism at a higher strength. Similar profiles were observed at 18 and 10°C. In general, SMP production increased with increasing feed strengths.

Normally, the SMP/SCOD ratio increased along the reactor as the organics were removed. However, SMP accounted for 50%–75% of the SCOD in the last chamber of the CABR, in the present investigation (Fig.2). This value was much lower than the previous values (75%–100%) (Barker *et al*., 2000). However, the authors' objective was to study the effect of a short shock on SMP production, hence, a shorter HRT was applied. Thus, the acetate consuming microorganisms adapted to the changing conditions slower than the fast growing acetate producing microorganisms.

UAP were produced as a result of the temporarily high organic loading (Aquino and Stuckey, 2004), and were more biodegradable than BAP. At 28°C, the SMP accounted for (4.20 ± 0.50)%, (9.19 ± 1.31)%, and (6.39 ± 0.36)% of the SCOD at feed strengths of 300, 450, and 600 mg/L, respectively. Kuo *et al*. (1996), found that the normalized production of SMP appeared to be 0.2%–2.5% of the influent COD, in anaerobic systems for cultures fed on glucose, and (14.7 ± 3.7)% for cultures fed on phenol (Boero *et al*., 1991).

2.2 Effect of the HRT

There was increased SMP production with decreasing HRT in the CABR as shown in Fig.3 and Table 3. An identical trend was observed in a previous study (Schiener *et al*., 1998), that is, the concentration of SMP increased from (23.80 ± 14.79) mg/L to (37.10 ± 19.72) mg/L as the HRT was decreased from 18 to 12 h and slightly decreased to (35.88 ± 9.98) mg/L at 9 h HRT.

A reasonable explanation is that the activity of microorganisms decreases to some extent as the operational temperature is reduced, however, it appears as if the partial consumption/mineralization of SMP occurs as a result of

Table 3 SMP of the CABR at different temperatures and HRTs (n = 3, mean ± SD, mg/L)

HRT (h)	Temperature			Average
	10°C	18°C	28°C	
9	42.16±8.83	41.10±0.93	24.37±0.79	35.88±9.98
12	52.22±6.07	44.29±1.41	14.79±1.21	37.10±19.72
18	40.57±3.18	18.22±1.30	12.61±1.49	23.80±14.79
Average	44.98±5.16	34.54±11.61	20.59±9.78	

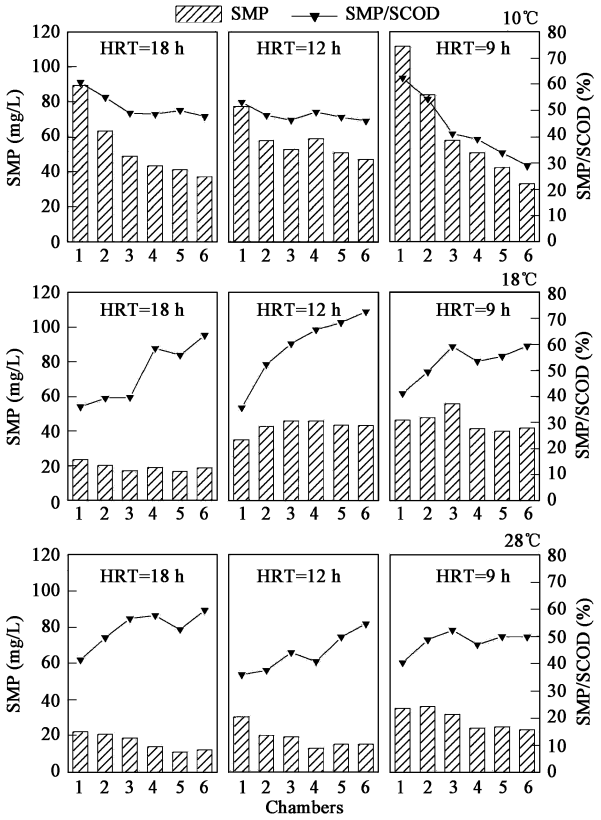

Fig. 3 SMP profile in the chambers at different temperatures and HRTs.

low feed strength at 18 h HRT. When the HRT is reduced to 9 h, the acidogenesis phase probably does not form, resulting in low SMP production because more SMP is produced during acidogenesis than during methanogenesis (Barker and Stuckey, 1999).

The ratios of SMPs at 18 and 9 h HRT in CABR were 1.93, 2.26, and 1.04 at 28, 18, and 10°C, respectively (Table 3). Hence, the effect of the HRT on SMP weakened gradually as the temperature decreased.

The SMP/COD ratio increased along the reactor, both at 28 and 18°C (Fig.3). However, there was a decrease at 10°C, because much of the SMP was comprised of nondegraded feed in the first two chambers, and the ratio gradually dropped as the feed gradually degraded along the reactor. A very high SMP concentration found in the first compartment of the reactor, at 9 h HRT, could probably be because of the presence of a nondegraded substrate that could not be measured.

Obviously, a high SMP/SCOD ratio was observed at longer HRT because of the complete degradation of VFA, and a lower ratio at shorter HRT was probably because of the incomplete degradation of the feed.

2.3 Effect of operational temperature

Generally, more SMP was observed at high temperature because of the complete degradation of VFA, but less SMP was detected at low temperature perhaps because of the incomplete degradation of the feed.

SMP production increased with decreasing temperature as is evident in Tables 2 and 3, which was in agreement with the previous studies (Barker *et al*., 2000; Nachaiyasit and Stuckey, 1997b; Schiener *et al*., 1998), although these studies were conducted using different feed types and strengths (Nachaiyasit and Stuckey, 1997b). It was suggested that at low temperature, the biomass was under stress and produced more SMPs to protect cells (Schiener *et al.*, 1998), and the bacterial metabolism slowed down, which led to a slower rate of substrate and SMP degradation.

Table 2 shows a comparison of SMP production at 18 h HRT for three different temperatures (10, 18, and 28°C). SMP productions at 28 and 18°C are quite similar, but very different from the production at 10°C. From the results, it is clear that low temperature greatly inhibits microbial growth, which results in an abrupt increase in SMP.

Table 3 shows that SMP production increased with decreasing HRT at 28°C. However, the SMP production peak was for 12 h HRT when the temperature was decreased to 18 and 10°C. In general, the SMP concentration increased with decreasing HRT. However, the authors' observations at 18 and 10°C were in contrast to the above theory, but agreed with the former studies of Barker *et al*. (2000). They postulated that such a difference could be a result of the initial biomass concentration in the reactor, whereas, the possible reason seemed to be the temperature in this investigation, that is, the temperature was an important factor in determining the relationship between HRT and SMP, although there was no definite experimental evidence provided in this study. Therefore, temperature could be an important factor in determining the SMP dependence on HRT.

3 Conclusions

SMP production increased with the increasing feed strengths and decreasing temperatures in the CABR. At high temperature (28°C), SMP production increased with decreasing HRT. The peak in SMP production was found for an HRT of 12 h when the temperature was decreased to 18 and 10°C. It was possible that some SMP could have been mineralized at 18 h HRT, whereas, the acidogenesis phase did not completely form at 9 h HRT, because more SMP was produced during acidogenesis than during methanogenesis.

A high SMP/SCOD ratio was found at high temperature and long HRT because of the complete degradation of VFA. SMP accounted for 50%–75% of the SCOD in the last chamber in this investigation, which was much lower than in the previous studies. The SMP/SCOD ratio increased along the reactor as the organic materials were removed.

Some SMPs, as secondary metabolites, could be consumed at a low feed strength. At a constant dilute feed strength (300 mg/L COD), the peak in SMP production for various applied HRTs occurred at 12 h and the SMP production decreased with the HRT decreasing to 9 h at lower applied temperatures (18 and 10°C). However, the SMP production increased at 28°C. Therefore, temperature could be an important factor in determining the SMP dependence on the applied HRT.

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