Carbon Fiber Amended Anaerobic Biofilm Reactor for Source-separated Blackwater Treatment

by

Qi Huang

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Department of Civil and Environmental Engineering
University of Alberta

Abstract

Decentralized domestic wastewater treatment is a new sanitation concept by separating greywater and blackwater at source for different treatment procedures. Anaerobic treatment is regarded as the core technology for simultaneous organic matter removal and biomethane production from source-separated blackwater, which possesses huge potential for bioenergy recovery. In this study, a promising treatment system, named carbon fiber amended anaerobic biofilm reactor, was designed and operated for evaluation of long-term performance and system stability for high-strength blackwater. At different hydraulic retention times (HRTs) ranging from 20 to 5 days and organic loading rates (OLRs) varied from 0.77 to 3.01 g COD/L-d in four stages, superior and stable performance was observed during the long-term operation about 250 days. With the increase of OLRs, the specific methane production rate increased from 105.3 to 304.6 mL/L-d with high purity of methane (75.5-83.0 %). The maximum methane yield was achieved at HRT of 15 days, which was 38.4% out of 45% biochemical methane potential (BMP). Highest organic compound and suspended solid removal (80-83 %) was achieved at 20-days HRT, while the increased OLRs resulting in diminished removal efficiencies. The state variables including pH, total ammonia nitrogen (TAN), short-chain volatile fatty acids (SCVFAs) and soluble COD (SCOD), indicated the system had a great capacity to withstand the high organic loading rates for anaerobic blackwater treatment.

Keywords: source-separation, blackwater, anaerobic digestion, conductive carbon fiber

Preface

The research findings in this thesis (Chapter 1, 3, 4, 5) will be submitted as Qi, Huang; Basem S., Zakaria; Yingdi, Zhang; Lei, Zhang; Yang, Liu; Bipro R., Dhar (2019) "Carbon fiber amended anaerobic biofilm reactor for source-separated blackwater treatment" to a journal for peer-review and publication. For this manuscript, Q. Huang was responsible for experimental design, laboratory experiments, data collection and analysis as well as the manuscript composition. B.S. Zakaria, Y. Zhang and L. Zhang assisted with the data collection. Y. Liu and B.R. Dhar planned and supervised the study. All authors contributed to the manuscript preparation.

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Chapter 1

Introduction

1.1 Background

Domestic wastewater from most urban areas around the world is collected and treated in centralized plants. Increasingly, decentralized wastewater treatment is being considered as a sustainable sanitation concept for urban water management, by separating blackwater and greywater at source for different treatment processes (Gallagher & Sharvelle, 2010). Blackwater, which is a mixture of feces, urine, toilet paper and flush water, accounts for less than 30% of the total volume but contains about 70% organic matter and 80% nutrients of domestic wastewater (Kujawa-Roeleveld & Zeeman, 2006; Moges et al., 2018). Therefore, blackwater separation at source and decentralized treatment of blackwater stand out in the ability to recover energy and nutrient resources from domestic wastewater. Anaerobic digestion (AD) is a technically feasible and cost-effective technique for source-separated blackwater treatment with simultaneous organic removal and bioenergy recovery, low excess sludge generation and smaller footprint (Aiyuk et al., 2006; Foresti, 2002; Lettinga et al., 2001). However, due to the complex composition and low biodegradability of blackwater, conventional anaerobic digestion of blackwater in previous studies were mostly operated at low organic loading rates (OLRs) ranging from 0.3 to 0.5 g COD/L-d (Kujawa-Roeleveld et al., 2006; Luostarinen & Rintala, 2007; Luostarinen et al., 2007; Luostarinen & Rintala, 2005; Meulman et al., 2008; Wendland et al., 2007; Zamalloa et al., 2013), while high-rate blackwater digestion was barely investigated.

In recent years, conductive materials have been intensively reported to enhance methane production in anaerobic digestion processes by promoting direct interspecies electron transfer (DIET) in microbial communities (Dang et al., 2017; Liu et al., 2012a; Lovley, 2017; Yan et al., 2017; Yang et al., 2017), which is a promising complementary to indirect electron transfer via intermediates like hydrogen or formate (Dong & Stams, 1995; Martins et al., 2018; Schmidt & Ahring, 1995; Thiele & Zeikus, 1988). The DIET between some specific electroactive bacteria and electrotrophic methanogens (Morita et al., 2011; Rotaru et al., 2014a; Rotaru et al., 2014b) allows rapid conversion of organics to methane with a higher electron transfer rate than conventional pathways (Barua & Dhar, 2017).

To date, there are only few studies on the effect of conductive material supplementation on blackwater digestion. (Florentino et al., 2019; Xu et al., 2019). Granular activated carbon (GAC) addition has been utilized in batch test for concentrated blackwater digestion with 19% higher biochemical methane potential (BMP) compared to control group (Florentino et al., 2019). The results also showed a microbial community shift towards syntrophic bacteria and methanogenic archaea, such as the enrichment of *Geobacteraceae* family and *Methanosarcina*, which likely participated in DIET (Florentino et al., 2019). Recently, the effect of nano-scale zero valent iron (nZVI) addition on anaerobic blackwater digestion at different dosages (i.e., 0.5, 1, and 10 g/L) was investigated by Xu et al. (2019). The results showed low dosage (0.5-1.0 g/L) of nZVI50 (50nm, > 80% Fe^0) increased the blackwater BMP to 42.9-45.2% compared to 36.8% of control group, with enhanced hydrolysis and acidification (Xu et al., 2019). However, these studies were conducted in batch mode, which has left open a wide research gap in understanding the impacts of conductive material addition on performance of different anaerobic reactors under continuous mode.

1.2 Specific objectives

The application of conductive materials in anaerobic digestion is considered as a potentially energy-conserving approach to improve methanogenesis rate. However, the application of conductive materials on anaerobic blackwater digestion in continuous reactors was hardly reported. Based on the aforementioned research gaps, the overall objective of this thesis was to evaluate performance and long-term stability of an anaerobic biofilm reactor amended with conductive carbon fibers. First, the BMP of vacuum toilet blackwater used in this study was evaluated over 90-days incubation at 20 °C. Meanwhile, a long-term performance (i.e. specific methane production rate, methane content, organic matter removal, and system stability) of the carbon fiber amended anaerobic biofilm reactor was investigated as hydraulic retention times (HRTs) decreasing from 20 to 5 days and OLRs increasing from 0.77 to 3.01 g COD/L-d. This study was conducted to provide valuable information and recommendations to further engineer the concept of conductive material assisted anaerobic digestion.

1.3 Thesis organization

This dissertation documented the performance of a carbon fiber amended anaerobic biofilm reactor treating vacuum toilet blackwater at different HRTs ranging from 20 to 5 days. The organization of this thesis is as follows. Chapter 2 provides a literature review on the the current status of anaerobic digestion studies on source-separated blackwater. The review pointed out the significance of anaerobic treatment of blackwater separated from domestic wastewater at source. Various anaerobic bioreactor systems for blackwater treatment were presented, compared and discussed in this review. Chapter 3 details the reactor configuration, experimental design, analytical methods and calculations throughout the study. Following this, Chapter 4 presents the results and discussions on the experimental work and data analysis. The blackwater treatment performance of the carbon fiber amended anaerobic biofilm reactor in terms of specific methane production rate, methane content, organic matter removal, and system stability was presented and interpreted in this section. Finally, Chapter 5 summarizes the conclusions from the long-term operation of the carbon fiber amended anaerobic biofilm reactor. Major performance parameters were pointed out as references for engineering practice. The recommendations for future work were also proposed in this chapter.

Chapter 2

Literature Review

2.1 Significance of decentralized anaerobic domestic wastewater treatment

Water is essential for the existence of life and keeping the world function well. Generally, each person on earth requires about 140 to 280 liters of clean, safe water per day for drinking, cooking, and washing (de la Cruz et al., 2017; Luan, 2010). Thus, safe, clean, and accessible water is a critical issue for public health, natural environment, and economic growth. The contaminants in produced wastewater or sewage by human activities are removed by various wastewater treatment technologies which convert contaminated water into cleaner effluent that can be returned to the water cycle or directly reused (Metcalf, 2003).

Conventional domestic wastewater treatment schemes employing aerobic activated sludge treatment and anaerobic sludge digestion are energy intensive, usually, 0.6 kWh/m^3 of wastewater treated, with about half of electrical energy used for the aeration (Curtis, 2010; McCarty et al., 2011). In most of the developed countries, the energy demands for wastewater treatment account for about 3% of the total electrical energy load (Curtis, 2010). Furthermore, large quantities of sludge are produced with low potential for resource recovery from wastewater using conventional aerobic treatment methods (Gu et al., 2016). The greenhouse gas (GHG) emission from aerobic microbial respiration and intensive power consumption in activated sludge process could not comply with the concept of sustainability (Gori et al., 2011; Stokes & Horvath, 2009). Based on the goal of the water-energy nexus in wastewater management, the focus on reducing energy demands while recovering energy and value-added resources (e.g., nutrients) from wastewater streams has been powerful drivers for innovations of domestic wastewater management (Guest et al., 2009).

Anaerobic digestion (AD) has been considered as a viable alternative of conventional aerobic processes for energy recovery in the form of biomethane, with great potential in achieving cost reduction and sustainability (Lettinga et al., 2001). In comparison to aerobic domestic wastewater treatment, anaerobic processes are net energy producing processes instead of energy consumption with no aeration needed. Besides, AD has lower nutrient and smaller footprint requirement and generates significantly lower sludge production (Aiyuk et al., 2006; Foresti, 2002; Lettinga et al., 2001). The produced biomethane can be employed in numerous end-use applications (McCarty et al., 2011): (1) on-site combustion for heat or electricity generation; (2) transported to a local natural gas provider, or (3) used as vehicle fuel. Therefore, from foregoing, the anaerobic domestic wastewater treatment offers the possibility of operation in energy neutral or even positive and cost-effective schemes. However, the low organic concentration of domestic wastewater is one of the major barriers to direct anaerobic treatment. Thus, decentralized domestic wastewater treatment was introduced, collecting most concentrated domestic wastewater streams separately at source for the anaerobic digestion (Kujawa-Roeleveld & Zeeman, 2006).

2.2 Source separation of blackwater

By applying source separation, efficient recovery of bio-energy from domestic wastewater can be achieved by anaerobically treating them separately according to the stream quality and quantity (Larsen et al., 2013; Zeeman & Kujawa-Roeleveld, 2011). Generally, household wastewater is divided into two main streams: blackwater and greywater (Fig.1). Greywater, mainly from showers, laundry, and kitchen, accounts for the largest portion (70%) by volume of total domestic wastewater while characterized by significantly lower concentrations of pollutants and nutrients compared to blackwater (Günther, 2000; Hernandez Leal et al., 2007).

Blackwater is a mixture of feces, urine, toilet paper and flush water, which contains about 70% of the organic matter and 80% of the nutrients in domestic wastewater, while accounting for less than 30% of the volume (Kujawa-Roeleveld & Zeeman, 2006; Moges et al., 2018). Meanwhile, human feces are the main source of pathogenic pollution, hormones, and pharmaceutical residues existing in wastewater (De Graaff et al., 2010). Therefore, source separation of blackwater from total domestic wastewater provides great potential for increased treatment efficiency, especially when using AD, which is a technically feasible and cost-effective option for high-strength wastewater stream treatment.

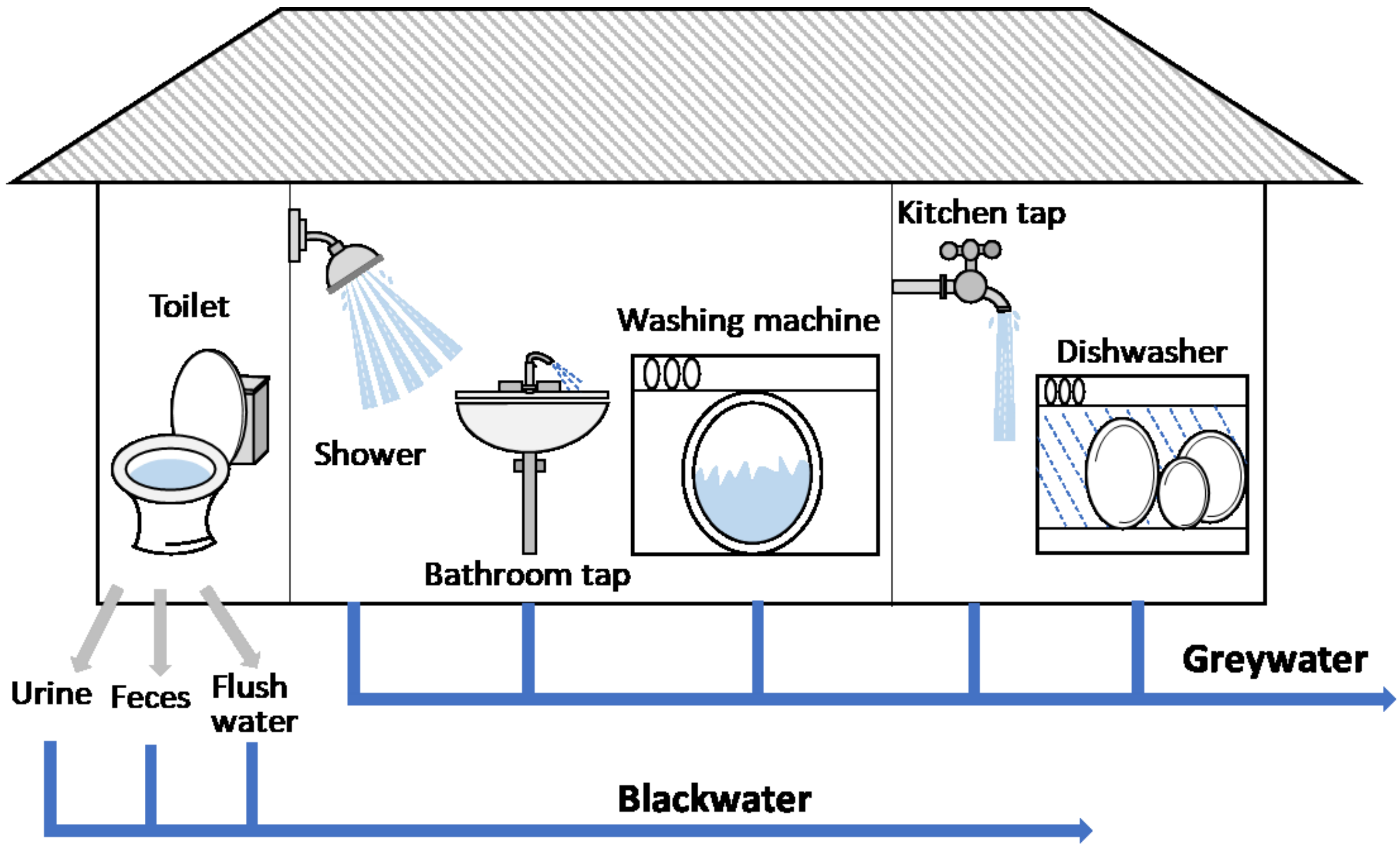


Fig. 1. Source separation of blackwater from domestic wastewater.

2.3 Blackwater collection system and characteristics

The concentration and characteristics of blackwater can be influenced by a collection system or a toilet type, as the flush water used in the toilet decides the dilution times of blackwater, resulting in variations in blackwater quality and quantity. Vacuum flush toilets use only 0.5–1.2 L water per flush with blackwater production of 5-7 L/cap/d, whereas dual flush systems use 3-6 L water per flush and conventional toilet flush systems use 9 L per flush (Gao et al., 2018a).

The major physicochemical properties of the blackwater in different studies are summarized in Table 1. The blackwater shows high pH level ranged from 8.4 to 8.8, which could be attributed to protein hydrolysis and high urea content (Cook et al., 2007; Gao et al., 2018b). Suspended COD accounts for the largest portion of total COD (TCOD) of 52–82 %, whereas soluble COD (SCOD) and colloidal COD was only 15–34 %, 2–13% of total COD, respectively.

Vacuum blackwater showed highest ammonia concentration of 0.7-1.4 g/L due to a high urine content. The co-existence of high pH and high ammonia concentration can lead to a high free ammonia (FA) concentration, which is known as a powerful inhibitor for methanogenesis process (Chen et al., 2008; Yenigün & Demirel, 2013). Thus, even though water consumption can be saved during collection and organics can be concentrated to achieve higher energy recovery efficiency in vacuum toilet collected blackwater treatment, high concentration of pollutants or inhibitory factors (e.g. ammonia and sulfide) inhibits the anaerobic processes with reduced treatability (Chen et al., 2014a; Chen et al., 2008; Gao et al., 2018a; Koster et al., 1986). Lowest biochemical methane potential (BMP) (34-60%) was achieved in vacuum toilet collected blackwater compared to other types of blackwater (Gao et al., 2018a; Kujawa-Roeleveld et al., 2006; Kujawa-Roeleveld et al., 2005; Wendland et al., 2007).

Table 1. Physicochemical properties of blackwater from previous studies.

Parameter (g/L)	Vacuum toilet (Gao et al., 2018a)	Dual toilet (Gao et al., 2018a)	Conventional toilet (Gao et al., 2018a)	Vacuum toilet Sneek (day 1-518) (De Graaff et al., 2010)	Vacuum toilet Sneek (day 519-951) (De Graaff et al., 2010)	Vacuum toilet UASB-ST1 (Kujawa-Roeleveld et al., 2005)	Vacuum toilet UASB-ST2 (Kujawa-Roeleveld et al., 2005)
pH	8.6	8.5	8.4	8.8 ± 0.22	8.6 ± 0.53	8.81 ± 0.2	8.65 ± 0.4
Total COD	29.25	4.71	2.58	9.8 ± 2.6	7.7 ± 2.5	9.503 ± 6.46	12.311 ± 7.782
Suspended COD	19.32	3.15	1.544	5.1 ± 2.7	4.9 ± 2.0	7.869 ± 6.138	9.653 ± 6.827
Soluble COD	8.88	1.545	0.888	3.4 ± 0.47	2.3 ± 0.81	1.433 ± 0.479	2.001 ± 1.209
Colloidal COD	1.320	0.060	0.148	1.3 ±0.42	0.5 ± 0.22	0.201 ± 0.160	0.658 ± 0.812
TAN	1.04	0.182	0.0964	1.4 ± 0.15	0.85 ± 0.15	0.708 ± 0.101	1.042 ± 0.096
TN	1.7	0.410	0.190	1.9 ± 0.19	1.2 ± 0.18	-	-
TP	0.330	0.0705	0.038	0.22 ± 0.067	0.15 ± 0.064	0.114 ± 0.063	0.144 ± 0.061
TS	17.14	3.57	2.39	-	-	-	-
VS	14.2	2.825	1.847	-	-	-	-

Note:

TAN: total ammonia nitrogen;

TN: total nitrogen;

TP: total phosphorus;

TS: total solid;

VS: volatile solid.

2.4 Various anaerobic bioreactor systems for blackwater treatment

To date, six types of anaerobic bioreactor systems (see Fig. 2), namely up-flow anaerobic sludge blanket (UASB), UASB-septic tank (UASB-ST), accumulation (AC) system, continuous stirred-tank reactor (CSTR), microbial electrolysis cell-septic tank (MEC-ST), and anaerobic baffled reactor (ABR) were used in blackwater treatment. The performances of these various anaerobic systems are summarized in Table 2.

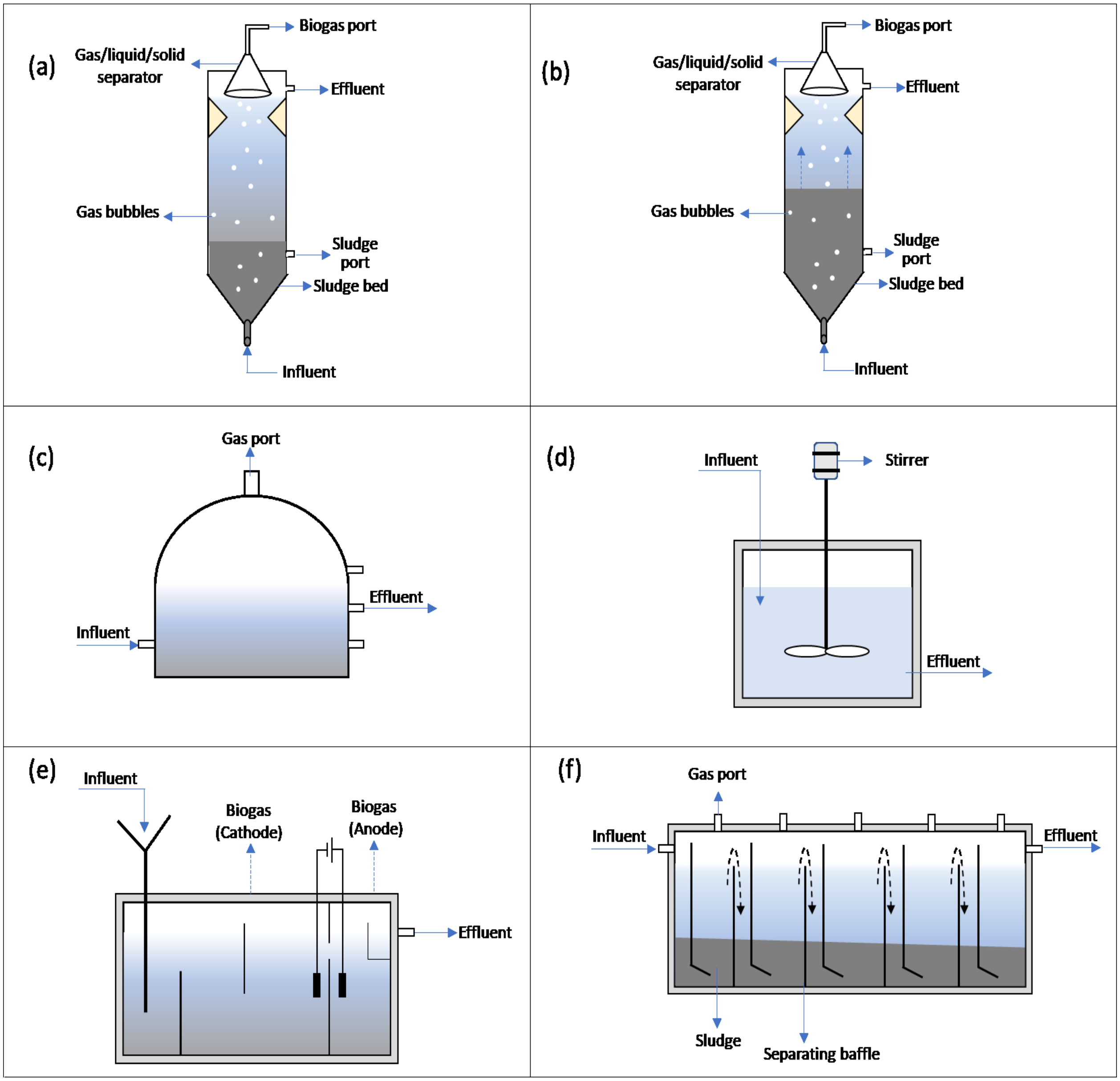


Fig. 2. Schematic diagrams of different anaerobic bioreactor systems studied for anaerobic blackwater treatment: (a) UASB; (b) UASB-ST; (c) AC; (d) CSTR; (e) MEC-ST; (f) ABR.

2.4.1 Up-flow anaerobic sludge blanket (UASB)

Up-flow anaerobic sludge blanket (UASB) reactor has been successfully utilized for the high-rate treatment of various wastewater streams. In UASBs, wastewater enters the reactor from the bottom through an influent distribution system, and travels upward through a dense anaerobic sludge bed, where soluble organic matters are digested and converted to biogas by the biomass granules while suspended organics are entrapped for subsequent biodegradation (Lettinga et al., 1980). UASB bioreactors provide long sludge retention times (SRTs) with good stability and high methanogenic activity in the sludge bed, which allows high OLRs, short HRTs, and low energy requirements (Daud et al., 2018).

UASBs have been intensively investigated for anaerobic blackwater treatment (De Graaff et al., 2010; Gallagher & Sharvelle, 2011; Gallagher & Sharvelle, 2010; van Voorthuizen et al., 2008). A 50 L flocculent sludge UASB reactor was operated at 25°C for vacuum toilets collected blackwater treatment (TCOD 7.7-9.8 g/L) by De Graaff et al. (2010). A short HRT of 8.7 days and an OLR of 1.0 g COD/L-d have been successfully achieved in this reactor, with 53% of the suspended solids hydrolyzed to methane and 78% TCOD removal (De Graaff et al., 2010). A 95 L UASB was operated at 28°C for diluted blackwater (TCOD ~ 0.9 g/L) treatment at HRT of 2.3 to 3.6 days and OLR of 0.23 to 0.45 g COD/L-d (Gallagher & Sharvelle, 2010). An average COD removal efficiency of 72% was achieved, with relatively low methane yield of 137 L CH_4/kg COD_{input}, which could be attributed to low influent COD concentration (Gallagher & Sharvelle, 2010).

van Voorthuizen et al. (2008) compared the performances of a UASB followed by effluent membrane filtration and an anaerobic MBR, for treating diluted blackwater (TCOD ~ 1.139 g/L) collected from school toilets. At 12 h HRT and 37°C in both UASB and anaerobic MBR, high total COD removal efficiencies were achieved, which were 91 and 86%, respectively. However, compared to BOD_5/COD ratio of 0.66, relatively low methane production of only 0.27 and 0.35 g CH_4-COD/g feed COD were observed respectively, which could be seriously underestimated. Besides, part of suspended and colloidal organics was washed out from the UASB reactor, resulting in the accumulation in the following membrane filtration step. The washed-out organics are no longer available for methane production, which led to lower methane production in the UASB reactor compared to the anaerobic MBR (van Voorthuizen et al., 2008).

UASB is one of the highly efficient reactors for anaerobic treatment and methane recovery, with scalability, simple construction, and small footprint (Gomec, 2010; Mao et al., 2015; Shoener et al., 2014). Higher organic loading rates for blackwater treatment in the UASB could possibly be achieved, which requires further investigation. However, treatment efficiency maybe unstable with variable organic loads in an UASB with relatively low resistant to shock loading (Kaviyarasan, 2014). Long start-up phase and constant electricity input for up-flow influent are required (Show et al., 2004). Furthermore, the effluent from UASB still requires further treatment to remove pathogens which are highly contained in blackwater (Kaviyarasan, 2014; Khan et al., 2011; Pant & Mittal, 2007; Paulo et al., 2013).

2.4.2 UASB-septic tank (UASB-ST)

Bogte et al. (1993) firstly investigated onsite domestic wastewater treatment using a variant of the UASB reactor, which is known as UASB-septic tank (UASB-ST). The sludge volume in a UASB-ST increases with time without regular sludge disposal, whereas the sludge bed in a UASB should be kept at a steady level. Therefore, the major feature of UASB-ST that distinguishes it from conventional UASB is that the sludge accumulation and stabilization is allowed in this system. Compared to the conventional septic tank system, it differs by the up-flow hydraulic mode, with an improved physical suspended solid removal as well as more efficient biological conversion (Adhikari & Lohani, 2019).

Most anaerobic blackwater treatment studies were performed with UASB-septic tanks (Kujawa-Roeleveld et al., 2006; Kujawa-Roeleveld et al., 2005; Luostarinen & Rintala, 2007; Luostarinen et al., 2007; Luostarinen & Rintala, 2005; Zeeman et al., 2008). Kujawa-Roeleveld et al. (2005) investigated the performance of the UASB-septic tank for the treatment of vacuum toilet blackwater under two different temperatures for a period of one year. Two pilot-scale UASB-septic tank reactors (UASB-ST1 and UASB-ST2), each with 0.2 m^3 working volume, were operated at 15 and 25 °C respectively. The average HRT was approximately 29 days, and due to the variations in the quality of the blackwater feeding, the OLRs were 0.33 and 0.42 g COD/L-d, respectively. UASB-ST1 obtained an average TCOD removal efficiency of 61% and suspended COD removal efficiency of 88%, while for UASB-ST2, 78% and 94% removal efficiency were achieved for TCOD and suspended COD, respectively. At the end of the experimental period, the average daily biogas production was only 6.4 and 8.3 L/d for UASB-ST 1 and 2, respectively, which could be possibly underestimated.

Luostarinen et al. (2007) investigated the performance of a 1.2 m^3 pilot-scale UASB-septic tank which had been operated for 13 years and could be assumed to be fully adapted to temperature variations of different seasons. Blackwater with an average TCOD concentration of 2.897 g/L was fed into the reactor at ambient temperatures of 14–19°C. The reactor achieved an average COD removal efficiency of 70% at an OLR of 0.89 g COD/L-d and an HRT of 4.1 d.

Two-phase UASB-septic tanks were also adopted for anaerobic blackwater treatment (Luostarinen & Rintala, 2007; Luostarinen & Rintala, 2005). Diluted blackwater with 1 g/L TCOD was mimicked by primary sludge, tap water, and toilet paper in these studies. It was suggested that two-phased UASB-septic tank was feasible for onsite treatment of synthetic blackwater (HRT 4.4 d+1.4 d; OLR 0.301 g COD/L-d) at a low temperature of 10-20°C, providing efficient solid and dissolved organic removal (Luostarinen & Rintala, 2007; Luostarinen & Rintala, 2005).

2.4.3 Accumulation systems (AC)

An accumulation (AC) system combines digestion and storage in a single reactor volume, which could allow having inflow without outflow with variable reactor volume (Elmitwalli et al., 2006). After reaching the maximum volume or the required storage time, the reactor is emptied or left for additional storage. Elmitwalli et al. (2006) investigated the treatment performance and feasibility of two AC systems for digestion of kitchen waste and vacuum toilet collected black water with (AC1) or without (AC2) urine. The average influent TCOD of the AC2 (38.835 g/L) was four times higher than the AC1 (TCOD ~ 9.996 g/L). After operation for 105 days at 20 °C, 58% removal of the TCOD was removed in both AC systems. However, a relatively high amount of particulate organics (30%) were settled in the bottom and remained in AC1 system, while only 8% remained in AC2 system. The results also showed higher hydrolysis, acidification and methanogenesis percentages in AC2 system (46%, 44%, and 53%, respectively) compared to those in AC1 system (22%, 19%, and 28%, respectively) (Elmitwalli et al., 2006). Elmitwalli et al. (2011) utilized the Anaerobic Digestion Model No.1 (ADM1) to evaluate the performance of the AC system treating concentrated blackwater. The model results suggested that the filling period longer than 150 days should be provided for obtaining a stable performance.

AC is a simple technique for management and operation with low cost, good stability, rich nutrients and huge potential for biogas production (El-Mashad et al., 2006), which can be employed with very low influent volumes due to the limitation on the reactor volume. Direct reuse of the AC effluent is possible because the long residence time of AC system allows high stabilization of digested steams and significant inactivation of pathogens (Kujawa-Roeleveld et al., 2006).

2.4.4 Continuous stirred tank reactor (CSTR)

Continuous stirred tank reactor (CSTR) is one of the most frequently used anaerobic systems without sludge retention, which is mainly utilized in the treatment of high strength wastewater streams (Luo et al., 2010). With continuous feeding, the mechanical blender of the CSTR provides liquid and suspended solids (including biomass) with the larger surface area for contact and reaction; thus, increasing gas production (Ohimain & Izah, 2017).

A CSTR of 10 L was operated at mesophilic temperature and fed with vacuum toilet blackwater (TCOD ~ 8.7g/L) (Wendland et al., 2007). At HRT of 20 days, the removal of total and particulate COD was 61% and 53% respectively, with methane production of

218.4 L/kg COD input. In spite of high ammonia concentration, the process was stable and uninhibited indicated by low concentrations of short-chain volatile fatty acids (SCVFAs) in the effluent (Wendland et al., 2007).

2.4.5 Microbial electrolysis cell-septic tank (MEC-ST)

So far, many researches have demonstrated that microbial electrochemical system coupled with anaerobic digester could considerably enhance methane productivity from high-strength wastewater streams (Bo et al., 2014; Cai et al., 2016; Gajaraj et al., 2017; Hobbs et al., 2018; Li et al., 2016; Sasaki et al., 2013). In a microbial electrolysis cell (MEC), hydrogen could be produced from the combination of protons and electrons released from the oxidation of substrate by exoelectrogens over thermodynamic barriers (Liu et al., 2005), which could enhance hydrogenotrophic methanogenesis with fast growth rate and stability at low temperature (Enright et al., 2009; Liu et al., 2016). Electro-methanogenesis via direct electron transfer from conductive cathode to electroactive methanogens can be another alternative pathway with fast methanogenesis rate (Cheng et al., 2009; van Eerten-Jansen et al., 2015; Zhen et al., 2015).

Zamalloa et al. (2013) investigated the performance of a laboratory-scale dual-chamber MEC integrated with a septic tank for the anaerobic treatment of concentrated blackwater (TCOD ~ 15.5 g /L) at OLR of 0.5 g COD/L-d and 30 °C. In the MEC-septic tank, a stable biogas conversion efficiency of about 30% was achieved during the above 100 days of operation time. The TCOD removal and the total suspended solid (TSS) removal were 85% and 90%, respectively. Interestingly, the H_2S concentration in the biogas output was 2.5 times lower, and the effluent phosphorus was 39% lower in the

MEC-septic tank than in the control septic tank. The phosphorus removal could be attributed to the phosphate precipitation on the cathode as struvite (Cusick & Logan, 2012). The H_2S could be removed by the sulfide oxidation by anodic microbes (Sun et al., 2009). Furthermore, the continuous corrosion of the anode results in the release of iron into the bulk solution, which could enhance the H_2S removal through the precipitation of iron sulfide (Nielsen et al., 2008; Pikaar et al., 2011).

2.4.6 Anaerobic baffled reactor (ABR)

The anaerobic baffled reactor (ABR) utilizes a series of baffles to force a wastewater stream to flow under and over (or through) the baffles when it passes from the inlet to the outlet (Barber & Stuckey, 1999). Flow characteristics and gas production force the sludge in the reactor to rise and settle gently (Shoener et al., 2014). Stability, reliability, and the solids retention capacity can be significantly enhanced in this design, which is ideal for high-strength wastewater treatment.

Moges et al. (2018) investigated the performance of an ABR for treatment of source-separated blackwater with an average TCOD of 5.5 g/L. Two identical reactors, each with a working volume of 16.4 L, were operated in parallel at 3-days HRT. The reactors were operated with different pulse lengths of 12 and 24 seconds per feed (114 L/h and 52 L/h) for the short-pulse fed reactor (RI) and the long-pulse fed reactor (RII), respectively. The results showed that concentrated blackwater was treated efficiently at 25-28 °C with TCOD removal efficiency kept stable above 78% at steady state. Biogas production ranged from 0.52 to 1.16 L/L-d, with 67–82% methane content in biogas and an average methanization of 69% and 73% for RI and RII, respectively. High OLR of 2.3 ± 0.5 g COD/L-d was achieved in the ABR with high methane conversion potential and organic content removal, which indicates that an ABR is capable of treating source-separated blackwater with high efficiency.

2.5 Co-digestion of blackwater and kitchen waste

Due to the different characteristics of wastewater streams, co-digestion may provide better carbon and nutrient balance with positive synergism established and therefore enhance the performance of the AD process (Mata-Alvarez et al., 2000). Kitchen waste (KW), the second highly concentrated stream from households, has been considered as a very attractive feedstock for anaerobic digestion due to its richness in nutrients and organics, high biodegradability and high methane potential (Zhang et al., 2013; Zhang et al., 2011). However, the accumulation of long-chain fatty acids (LCFAs) after fast hydrolysis could be inhibitory and toxic for syntrophic acetogens and methanogens (Hanaki et al., 1981), which can be alleviated by co-digestion of kitchen waste and blackwater. Studies also demonstrated that adding kitchen waste could boost methane production from blackwater digestion (Kujawa-Roeleveld et al., 2006).

The theoretical organic load of blackwater and kitchen waste per person is about 122 g COD/p-d (assuming faeces = 50, urine = 12, kitchen refuse = 60 g COD/p-d) (Kujawa-Roeleveld et al., 2006). Based on typical methanization rate of 60%, the methane production would be 28 L/p-d at 25 °C. However, the batch experiment demonstrated higher anaerobic biodegradability of 70-80% could be achieved in the mixture of blackwater and kitchen waste. Thus, an optimized digestion process could achieve higher methane production of 33-37 L/p-d (Kujawa-Roeleveld et al., 2006).

Wendland et al. (2007) investigated co-digestion of blackwater (5.0 L/d) and kitchen waste (0.2 g/d) in a CSTR with 10 L working volume at mesophilic temperature and different HRTs. At 20 days HRT, the addition of kitchen waste improved the COD removal efficiency and methane yield to 71% and 67%, respectively, compared to 61% and 53% in mono-digestion of blackwater under the same conditions. At HRT of 15 days, the co-digestion achieved the highest COD removal of 75% and the highest methane yield of 280 L/kg COD input with stable and uninhibited performance. However, when HRT decreased to 10 days, SCVFAs were accumulated in the system with decreased COD removal efficiency and methane yield. The results revealed under this scheme of co-digestion of blackwater and kitchen waste, an HRT of 15 days was recommended (Wendland et al., 2007). Comparable results were achieved in other studies for kitchen waste and blackwater co-digestion, which are shown in Table 3.

2.6 Enhanced anaerobic blackwater treatment using conductive additives

Traditionally, H_2 and formate are mostly used intermediates for interspecies electron transfer between fermentative bacteria and methanogens (Dong & Stams, 1995; Schmidt & Ahring, 1995; Thiele & Zeikus, 1988). However, recent research discovered direct interspecies electron transfer (DIET) could happen between some specific electroactive bacteria and electrotrophic methanogens (Morita et al., 2011; Rotaru et al., 2014a; Rotaru et al., 2014b), which allows rapid conversion of organics to methane (Barua & Dhar, 2017). Conductive materials have been added to digesters to promote DIET, thus improving AD efficiency, accelerating the conversion of organic matters to methane (Dang et al., 2017; Liu et al., 2012a; Lovley, 2017; Yan et al., 2017; Yang et al., 2017). Recently, granular activated carbon (GAC) addition has been utilized to overcome ammonia inhibition in methanogenesis process, resulting in 19% higher biochemical methane potential of concentrated blackwater in batch mode (Florentino et al., 2019). The addition of conductive material also resulted in a microbial community shift towards syntrophic bacteria and methanogenic archaea, such as the enrichment of *Geobacteraceae* family and *Methanosarcina*, which likely participated in DIET (Florentino et al., 2019).

In previous studies, zero valent iron (ZVI) has been reported to improve methane production in AD process involving both methanogenesis and hydrolysis-acidification processes (Feng et al., 2014). The ZVI can lower the oxidation–reduction potential (ORP) of substrate (Meng et al., 2013; Wei et al., 2018) and enrich hydrogenotrophic methanogens by directly providing electrons or producing hydrogen via the anaerobic iron oxidation (Karri et al., 2005; Meng et al., 2013), which offers favorable conditions for methanogenesis. In addition, the supplementation of ZVI can also accelerate the rate-limiting hydrolysis process (Liu et al., 2015; Liu et al., 2012b; Wang et al., 2018) and optimize the acidification by stimulating propionate degradation to acetate (Liu et al., 2015; Liu et al., 2012b; Suanon et al., 2017). Recently, Xu et al. (2019) investigated the effect of nano-scale zero valent iron (nZVI) addition on anaerobic blackwater digestion at different dosages (i.e., 0.5, 1, and 10 g/L). The results showed low dosage (0.5-1.0 g/L) of nZVI50 (50nm, > 80% Fe^0) increased the blackwater BMP to 42.9-45.2%, while control group only achieved 36.8% BMP. The study also indicated that the hydrolysis and acidification were enhanced by nZVI addition, which provided favorable substrate for methanogenesis (Xu et al., 2019). However, high dosage (10 g/L) of nZVI induced a pH increase above 8.5 resulting in higher free ammonia inhibition with deteriorative performance (Xu et al., 2019).

In summary, conductive additives possess great potential in promoting AD efficiency and methane recovery of blackwater via various mechanisms. However, since these studies were conducted in the batch mode, the impact of functional material addition on blackwater treatment efficiencies in different amended anaerobic reactors under continuous mode still needs to be evaluated.

Table 2. An overview of operational parameters and performances of blackwater mono-digestion in various anaerobic bioreactors.

Parameters	UASB (De Graaff et al., 2010)	UASB (Tervahauta et al., 2014)	UASB (Gallagher & Sharvelle, 2010)	UASB (Gallagher & Sharvelle, 2011)	Two-phase UASB-ST (Luostarinen & Rintala, 2005)	Two-phase UASB-ST (Luostarinen & Rintala, 2007)		UASB-ST (Luostarinen et al., 2007)	UASB-ST (Kujawa-Roeleveld et al., 2006)	UASB-ST (Meulman et al., 2008)	CSTR (Wendland et al., 2007)	ABR (Moges et al., 2018)	MEC-ST (Zamalloa et al., 2013)
Temperature (℃)	25	25	28	28	10	20	10	14-19	25	25	37	25-28	30
Reactor volume (L)	50	50	95	114	12+3	12+3		1200	200	7200	10	16.4	24.2
Average influent TCOD concentration (g/L)	7.7-9.8	7.1	0.9	0.932	1.057	1.046	1.161	2.897	9.5-12.3	16.1	8.7	5.5	15.5
HRT (d)	8.7	9.3	2.3-3.6	2.6-4.0	4.2+1.4	2.9+1.3		4.1	27-29	30	20	3	20-40
SRT (d)	254	138	-	-	-	-		-	> 365	> 365	20	-	-
OLR (g COD/L-d)	1.0	0.9	0.23-0.45	0.21-0.39	0.301+0.071	0.368+0.172	0.406+0.180	0.89	0.33-0.42	0.36	0.44	2.3	0.49
TCOD removal (%)	78	90	72	72	94	91	92	70	78	87	61	78	85
Methane production (L/p-d)	10	18.1	-	-	-	-		-	14.5	13	9.0	-	-
Methane yield (L/kg COD_{input})	211.8	210	137	105.9	-	-	-	66.9	170.7	124.2	240	267.7	113.2

Table 3. Characteristics of blackwater and kitchen waste mixture and performances of different anaerobic co-digestion systems.

Parameters	CSTR (Wendland et al., 2007)			AC (Elmitwalli et al., 2006)		AC (Kujawa-Roeleveld et al., 2006)	Two-phase UASB-ST (Luostarinen & Rintala, 2007)	
TCOD (g/L)		19.2		18.668	53.642	13.3-22.9	1.888	2.268
SCOD (g/L)		6.8		2.76	10.148	2.7-5.4	0.387	0.380
TAN (g/L)		1.15		0.586	0.785	0.6-1.3	0.0048	0.0064
TP (g/L)		0.171		-	-	0.11-0.21	0.017	0.016
Temperature (℃)		37		20		20	20	10
Reactor volume (L)		10		1220		1000	12+3	12+3
HRT (d)	20	15	10	105		115-150	3.4+1.3	3.4+1.4
OLR (g COD/L-d)	0.96	1.28	1.92	-	-	0.3	0.56+0.316	0.617+0.460
TCOD removal (%)	71	75	50	58	58	75.1	88	91
Methane production (L/p-d)	27	28	21	-	-	32.6-37.2	-	-
Methane yield (L/kg COD_{input})	270	280	205	198.8	105	267-304	-	-

2.7 Summary

To date, only limited research has been conducted to evaluate the application of anaerobic systems for blackwater treatment.

Concentrated blackwater collected from low-flush toilet or vacuum toilet usually contains high nutrient loads (Florentino et al., 2019; Gao et al., 2018a), which makes free ammonia inhibition a major challenge for blackwater digestion, especially under high pH and high temperature conditions, leading to suppressive methane production (Chen et al., 2008; Yenigün & Demirel, 2013). Therefore, focus on mitigating ammonia inhibition is required in future system design and process optimization.

Unlike greywater, which is usually discharged at mesophilic temperatures, blackwater is mostly discharged at room temperature to a decentralized treatment system (Kujawa-Roeleveld & Zeeman, 2006; Oteng-Peprah et al., 2018). The methanogenic microbiome is very sensitive to the operating temperature (Chae et al., 2008; De Vrieze et al., 2015; Levén et al., 2007). Thus, potential techniques to improve AD efficiency of blackwater at low temperatures need to be discovered and upgraded.

Due to the complex composition of blackwater as well as high suspended solids content, hydrolysis is the major rate-limiting step requiring long HRTs (Florentino et al., 2019; Vavilin et al., 2008). From previous studies, the OLRs mostly ranged from 0.3-0.5 g COD/L-d (Kujawa-Roeleveld et al., 2006; Luostarinen & Rintala, 2007; Luostarinen et al., 2007; Luostarinen & Rintala, 2005; Meulman et al., 2008; Wendland et al., 2007; Zamalloa et al., 2013). Thus, the development of innovative anaerobic bioreactor systems is required to promote high-rate blackwater treatment at shorter HRTs (i.e., higher OLRs).

Chapter 3

Methodology

3.1 Reactor configuration

The carbon fiber amended anaerobic biofilm reactor was fabricated in the laboratory using plexiglass tubes, with a total volume of 410 ml and a working volume of 330 ml. High density carbon fibers (2293-A, 24A Carbon Fiber, Fibre Glast Development Corp., Ohio, USA) were attached to a pair of stainless-steel frames, which were fixed on the left and right wall of the reactor, respectively. Before fabrication, the carbon fibers were pretreated for 3 days as described in a previous research (Dhar et al., 2013) with nitric acid (1 N), acetone (1 N) and ethanol (1 N) for 1 day in series, and then washed with ultrapure water (18.2 MΩ-cm). The reactor was continuously mixed by a magnetic stirrer and was equipped with one liquid sampling port and one gas outlet port connected to a gas bag for biogas collection. The configuration of the carbon fiber amended anaerobic biofilm reactor was shown in Fig. 3.

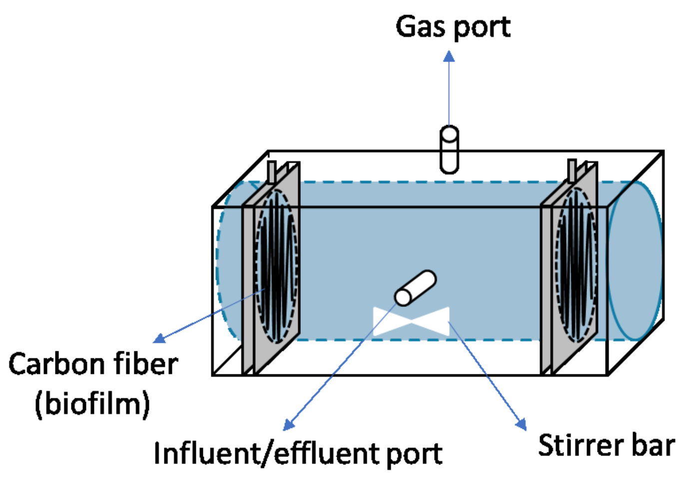


Fig. 3. Configuration of carbon fiber amended anaerobic biofilm reactor.

3.2 Inoculation and enrichment

The reactor was initially inoculated with 40 ml anaerobic digested sludge obtained from a lab-scale anaerobic digester with 25 g/L total suspended solid (TSS) and 13 g/L volatile suspended solid (VSS), 30 mL effluent from a dual-chamber microbial electrolysis cell (MEC) that had been operated with 25mM acetate medium for over 10 months, and 60 ml raw blackwater collected from vacuum toilet with details provided in 3.3.

For the enrichment of functional biofilms, the reactor was fed with synthetic blackwater for 4 months in sequencing batch mode. The composition and characteristics of the synthetic blackwater were provided in Table 4 and Table 5, respectively. The HRT was maintained at 14 days with an OLR of 1.875 g COD/L-d. The reactor was purged with nitrogen for 5 min to eliminate oxygen at the beginning of the experiment and incubated at room temperature (20 ± 0.5 °C).

Table 4. Chemical composition of the synthetic blackwater.

Substance	g/L
Starch $(C_6H_{10}O_5)_n$	13.70
Bovine serum albumin	4.00
Oleic acid ($C_{18}H_{34}O_2$)	0.70
$NaHCO_3$	5.00
NH_4Cl	3.82
KCl	0.02
Urea (CH_4N_2O)	0.28
$Na_2HPO_4 \cdot 7H_2O$	2.85
Trace elements-DSMZ 141	1 ml

Table 5. Characteristics of the synthetic blackwater.

Parameters	Value
TCOD	26.25 g/L
TAN	1.0 g/L
TP	0.33 g/L
TN	1.77 g/L
pH	8.6

Note: units in g/L except for pH.

3.3 Experimental start-up

3.3.1 Blackwater collection and characterization

Blackwater stock (feces and urine) was collected without flush water and toilet paper from healthy children, adults, and seniors on the University of Alberta campus using toilet waste bags. The collected blackwater stock was well mixed by a blender and stored at 4°C before further experiments. Blackwater stock was diluted using DI water to simulate vacuum toilet blackwater (1 L water per flush). The characteristics of raw blackwater used as substrate in this research are shown in Table 6.

Table 6. Physicochemical characteristics of vacuum toilet raw blackwater.

Parameters	Value
TCOD	15.2 ± 0.9
SCOD	5.2 ± 0.4
TAN	1.08 ± 0.08
TSS	6.29 ± 0.23
VSS	5.69 ± 0.14
pH	8.6 ± 0.1

Note: units in g/L except for pH.

3.3.2 Biochemical methane potential (BMP) tests

Initially, BMP tests were performed with vacuum collected (1 L/flush) blackwater in 157 mL serum bottles. Blank group was filled with the same amount of tap water and inoculum as blackwater and inoculum in test groups, respectively, to measure biogas generation from the inoculum. All the BMP tests were conducted in triplicate at 20°C in a shaker incubator (120 rpm) under dark conditions. No additional trace elements were added which were considered to be sufficient in the blackwater samples. After the addition of inoculum and blackwater, the serum bottles were flushed with nitrogen gas for 1 min and then sealed with a butyl rubber stopper and an aluminum cap. Biogas generation was monitored though regularly measuring the gas composition and the headspace pressure of the serum bottles.

3.3.3 Reactor operation

The carbon fiber amended anaerobic biofilm reactor was operated in semi-continuous mode at 20 ± 0.5 °C as follows: every certain days (the frequent varied in different stages), a predetermined volume was discharged and the same amount of fresh raw blackwater was added though the liquid sampling port. The stirrer bar was kept working during the effluent discharging and influent feeding process without solids settling in the reactor. A gas bag filled with nitrogen gas was connected to the gas sampling port during liquid discharging process to avoid oxygen intrusion into the reactor. From day 0 to 48 (stage 1), the reactors were operated at an HRT of 20 days with an average OLR of 0.77 g COD/L-d. From day 49 to 105 (stage 2) and day 106 to 180 (stage 3), the OLR was increased in stepwise fashion to 1.03 and 1.68 g COD/L-d respectively, by reducing the HRT to 15 days and 9 days respectively. Finally, from day 181 to 239 (stage 4), the HRT was shortened to 5 days with highest OLR of 3.01 g COD/L-d. The liquid was completely replaced with new substrate while the suspended biomass was kept inside the reactor every time when changing stage.

3.4 Analytical methods

The effluent samples were diluted and filtered with 0.45 μm membrane syringe filter for SCOD and TAN analysis. The TCOD and SCOD concentration was measured according to the standard methods using the closed reflux titrimetric method 5220C. TAN concentration was measured using Hach ammonia reagent kits (High Range, 0–50 mg nitrogen/l; Hach Co., Loveland, Colorado, USA). TSS and VSS were determined according to a standard method described in Federation and Association (2005). pH was measured using a B40PCID pH meter (VWR, SympHony). Biogas composition was measured using a 7890B gas chromatograph (Agilent Technologies, Santa Clara, USA) equipped with two columns (Molsieve 5A 2·44m 2mm for methane and HayeSep N 1·83m 2mm for oxygen, nitrogen, and carbon dioxide gases) and a thermal conductivity detector. The headspace pressure of serum bottles was measured using a GMH3151 manual pressure meter (Greisinger, Regenstauf, Germany). After diluted with ultrapure water and filtered using 0.2 μm membrane syringe filter, the SCVFA concentration of the effluent was measured with a Dionex ICS-2100 ionic chromatography system (Thermo Fisher, Waltham, MA, USA).

3.5 Calculations

Methane production in BMP tests was calculated using Equation (1) (Gao et al., 2018a):

$$CH_{4t} = \frac{64 \cdot P_t \cdot C_t \cdot V_h}{R \cdot T} \qquad (1)$$

Where:

CH_{4t}: Amount of methane production at time t (in mg COD);

P_t: Absolute headspace pressure at time t (in kpa);

C_t: Methane composition in the headspace at time t (in %);

V_h: Volume of headspace in serum bottles (in mL);

R: Gas law constant (in L kpa K^{-1} mol^{-1});

T: Absolute temperature (in K);

64: Conversion factor of 1 mol methane to 64 g COD.

Methane yield (%) was calculated using Equation (2) (Gao et al., 2018a):

$$Methane\ yield\ (\%) = \frac{COD_{methane}}{COD_{input}} * 100 \qquad (2)$$

Where:

$COD_{methane}$: The COD equivalent of produced methane (in mg COD);

COD_{input}: Amount of total COD input (in mg COD).

The free ammonia concentration was calculated using Equation (3) (Hansen et al., 1998):

$$NH_3(FA) = 1.214 \times TAN \cdot \left(1 + \frac{10^{-pH}}{10^{-(0.09018 + \frac{2729.92}{T\ (K)})}}\right)^{-1} \qquad (3)$$

Where:

NH_3: Free ammonia (FA) (in mg L^{-1});

TAN: Total ammonia nitrogen (in mg L^{-1});

T (K): Kelvin temperature.

COD removal was calculated using Equation (4):

$$COD\ removal\ efficiency\ (\%) = \frac{(COD_{input} - COD_{output}) * 100}{COD_{input}} \quad (4)$$

Where: COD removal efficiency (%)

COD_{input} = COD load fed to the reactor (g)

COD_{output} = COD load discharged from the reactor (g)

TSS removal was calculated using Equation (5):

$$TSS\ removal\ efficiency\ (\%) = \frac{(TSS_{input} - TSS_{output}) * 100}{TSS_{input}} \quad (5)$$

Where: TSS removal efficiency (%)

TSS_{input} = TSS load fed to the reactor (g)

TSS_{output} = TSS load discharged from the reactor (g)

VSS removal was calculated using Equation (6):

$$VSS\ removal\ efficiency\ (\%) = \frac{(VSS_{input} - VSS_{output}) * 100}{VSS_{input}} \quad (6)$$

Where: VSS removal efficiency (%)

VSS_{input} = VSS load fed to the reactor (g)

VSS_{output} = VSS load discharged from the reactor (g)

Chapter 4

Results and Discussions

4.1 Characterization of biochemical methane potential (BMP) of blackwater

Initially, maximum methane potential of vacuum toilet blackwater (1 L/flush) was measured from a long-term batch BMP test (Fig. 4). The batch experiment lasted for 90 days until methane production completely stopped. The BMP value for vacuum toilet blackwater in this experiment was about 45%, which is comparable with the biodegradability values (46-60 %) of vacuum toilet blackwater reported in previous studies (De Graaff et al., 2010; Kujawa-Roeleveld et al., 2006). However, the BMP value of concentrated blackwater in this study was relatively higher compared to 34% in a previous study (Gao et al., 2018a), which could be mainly ascribed to the lower incubation temperature (20°C) used in this study compared to 35 °C utilized by Gao et al. (2018a). Also, substrate to inoculum ratio could influence the estimated BMP (Pellera & Gidarakos, 2016; Yoon et al., 2014). Free ammonia (FA) concentration is primarily determined by three parameters such as pH, temperature and TAN (Rajagopal et al., 2013). Higher temperature and pH conditions usually lead to higher FA levels, which is considered as the main cause of inhibition of methanogenic communities (Fernandes et al., 2012; Ho & Ho, 2012). Thus, relatively high FA concentration (393 mg/L) was observed by Gao et al. (2018a) at 35°C, resulting in lower BMP value, while 11% higher BMP was achieved in this study at 20°C and FA concentration of 171.85 mg/L with higher digestibility and lower inhibition potentials. The results indicated that anaerobic digestion at lower temperature could expand the methane recovery potential of concentrated blackwater.

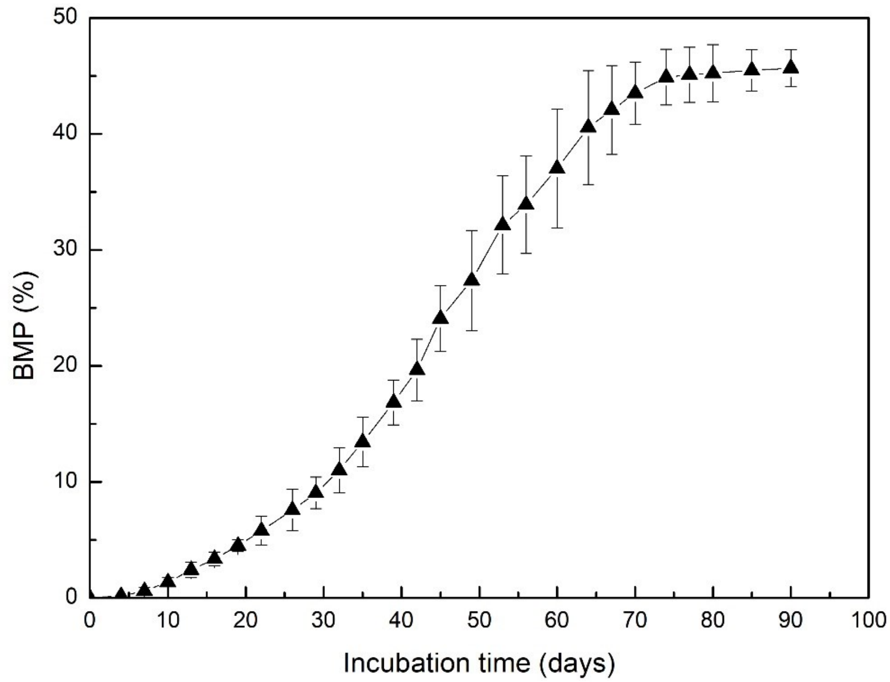


Fig. 4. BMP of vacuum toilet blackwater at 20 °C.

4.2 Biogas production in carbon fiber amended anaerobic biofilm reactor

A stable performance of the bioreactor at different OLRs was observed during the whole operation period of about 250 days. Based on the results of the steady state of each stage, 33.8 ± 1.3 % of methane yield (the ratio of COD equivalent of methane produced to the total COD input) was achieved in stage 1 at 20-days HRT (Fig. 5a). Interestingly, the methane yield increased to 38.4 ± 4.2 % in stage 2 at HRT of 15 days, then similar methane yield of 34 ± 2.3 % was observed in stage 3 at HRT of 9 days compared to stage 1. In stage 4 at HRT of 5 days and OLR of 3.01 g COD/L-d, the reactor exhibited the lowest methane yield of 27.1 ± 1.7 %. Based on the 45% BMP value of blackwater, 75.1%, 85.3%, 75.6%, 60.2% of biodegradable COD has been recovered as methane at HRT of 20, 15, 9, 5 days respectively. At the longest HRT in stage 1, the reactor showed lower methane yield than that of stage 2 probably because the electron portion utilized for microbial maintenance and growth increased at a low OLR. This phenomenon is consistent with previous studies which observed lower methane yield at longer HRTs (Feng et al., 2018a; Song et al., 2016). In addition, more biomass grew and retained on the biofilm gradually with time could also result in the higher methane yield in stage 2.

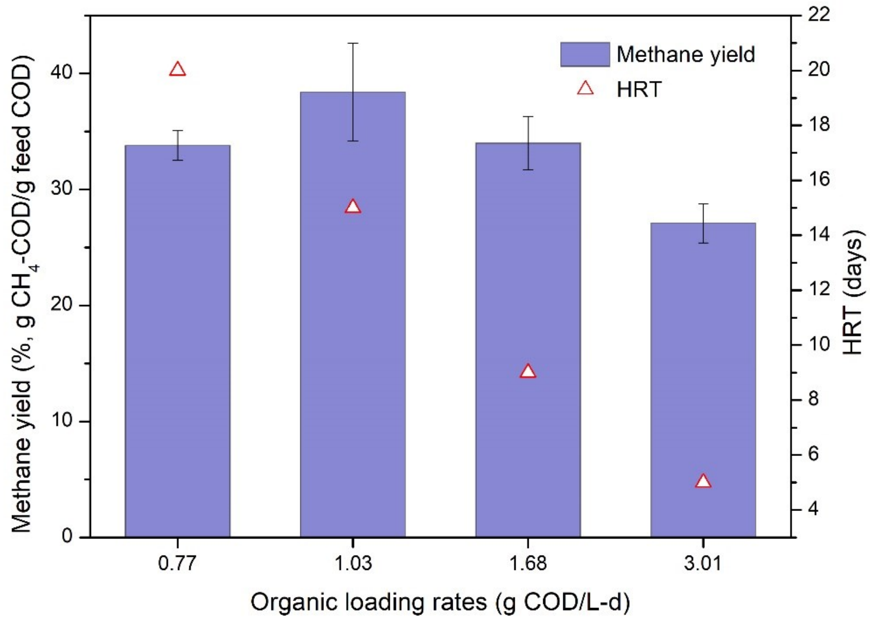
The total biogas production in the carbon fiber amended anaerobic biofilm reactor increased consistently with the HRTs decreased from 20 to 5 days in 4 stages, while the biogas composition was relatively stable (Fig. 5b). The specific methane production rate of steady state at 20-days HRT was 105.3 mL/L-d, which was significantly higher than that from some conventional anaerobic bioreactors investigated for blackwater treatment (31-73 mL/L-d) (Gallagher & Sharvelle, 2011; Gallagher & Sharvelle, 2010; Kujawa-Roeleveld et al., 2006; Kujawa-Roeleveld et al., 2005; Zamalloa et al., 2013). Due to the increase in organic loading rates, the specific methane production rates at steady state increased to 162.5, 211.0, and 304.6 mL/L-d at HRT of 15, 9, and 5 days, respectively.

The performance comparison of this study with previous studies was shown in Table 7. In previous studies, the OLRs were mostly ranged from 0.3 to 0.5 g COD/L-d (Gallagher & Sharvelle, 2011; Gallagher & Sharvelle, 2010; Kujawa-Roeleveld et al., 2006; Luostarinen & Rintala, 2007; Luostarinen et al., 2007; Luostarinen & Rintala, 2005; Meulman et al., 2008; Wendland et al., 2007; Zamalloa et al., 2013), including some studies performed under mesophilic conditions (Gallagher & Sharvelle, 2011; Gallagher & Sharvelle, 2010; Wendland et al., 2007; Zamalloa et al., 2013). However, in this study, high OLR of 3.01 g COD/L-d was achieved with a stable performance at 20°C.

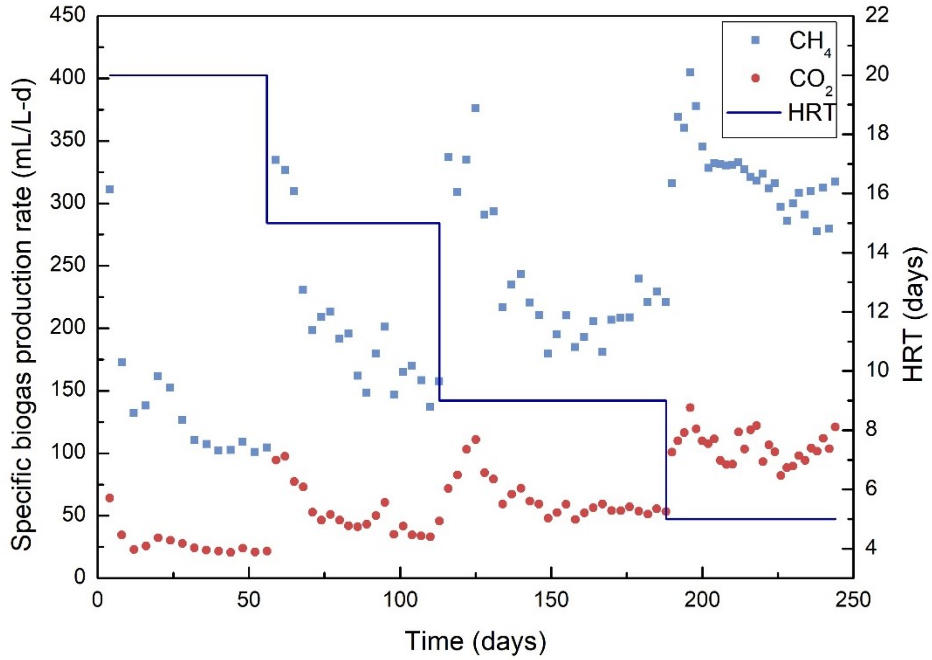
The high performance of the anaerobic biofilm bioreactor could be attributed to the following mechanisms: (i) the supplementation of conductive carbon fiber which served as the electron shuttle for the direct electron exchange, promoted the DIET between exoelectrogens and electroactive methanogens (Martins et al., 2018). The methane production was accelerated via DIET-based interspecies electron transfer with an 8.6 folds higher rate than interspecies H_2 transfer rate (Barua & Dhar, 2017; Storck et al., 2016); (ii) the addition of conductive materials in anaerobic bioreactors could possibly promote syntrophic partnerships DIET between exoelectrogens and hydrogenotrophic methanogens (Barua & Dhar, 2017; Florentino et al., 2019; Lee et al., 2016; Lin et al., 2017; Ryue et al., 2019; Sultana et al., 2015), resulting in a methanogenic pathway towards hydrogenotrophic methanogenesis, which is considered to be more tolerant to ammonia inhibition than acetoclastic methanogenesis (Chen et al., 2008; Florentino et al., 2019); (iii) The carbon fiber provided a large surface area for microbes to attach and grow, which remediated the short SRTs of conventional CSTRs, as the biomass retention on the biofilm could contribute to enhanced digestion efficiency and system stability (De Vrieze et al., 2014). Recently, many studies have reported the enhancement of methane production by the addition of conductive materials, including some carbon-based materials, such as graphite (Dang et al., 2016; Zhao et al., 2015), GAC (Dang et al., 2017; Lee et al., 2016; Liu et al., 2012a; Rotaru et al., 2014a; Xu et al., 2015; Yan et al., 2017; Yang et al., 2017; Zhang et al., 2017; Zhao et al., 2017a; Zhao et al., 2016b), carbon felt (Dang et al., 2016; Xu et al., 2016), biochar (Chen et al., 2014c; Shen et al., 2015; Zhao et al., 2016a; Zhao et al., 2015) and carbon cloth (Chen et al., 2014b; Dang et al., 2016; Dang et al., 2017; Lei et al., 2016; Zhao et al., 2017b; Zhao et al., 2015). Reduced lag phases and increased methane production rates have been observed in batch experiments with addition of conductive materials (Chen et al., 2014b; Dang et al., 2017; Liu et al., 2012a; Rotaru et al., 2014a; Salvador et al., 2017; Yan et al., 2017; Zhang & Lu, 2016). It has also been demonstrated in continuous anaerobic bioreactors that the conductive materials can enhance methane production and allow higher organic loading rates while maintaining reactor stability (Lei et al., 2016; Martins et al., 2018; Xu et al., 2015; Zhao et al., 2017a; Zhao et al., 2016a; Zhao et al., 2015), which is consistent with the results of this study.

The average methane content in biogas during the entire operation was >75%. As shown in Fig. 5c, the highest methane content of 83.0% was achieved in stage 1 at 20-days HRT, which was remarkably higher than that of conventional anaerobic digester for blackwater (62-78 %) (De Graaff et al., 2010; Gallagher & Sharvelle, 2011; Gallagher & Sharvelle, 2010; Kujawa-Roeleveld et al., 2006; Kujawa-Roeleveld et al., 2005; Zamalloa et al., 2013), then slightly decreased to 79.6% at 15-days HRT in stage 2. Lowest methane content of 75.5% was observed at HRT of 5 days, which was still higher than most of the conventional anaerobic bioreactors treating blackwater. The methane production in conventional anaerobic digestion mostly originates from indirect electron transfer via intermediates like acetate, hydrogen, and formate, which exhibited a high electron transfer loss (Feng et al., 2018a; Feng et al., 2018b; Zhao et al., 2016a). However, the conductive material addition could promote the DIET pathway and thereby enhance CO_2 reduction to provide higher methane content (Martins et al., 2018). Moreover, the possibly enriched hydrogenotrophic methanogens by conductive carbon fiber could further reduce carbon dioxide to methane (Barua & Dhar, 2017; Florentino et al., 2019; Ryue et al., 2019). The high pH in the solution could be another possible reason for high methane content, as the decreasing trend of methane content was consistent with the pH changes which decreased from 7.6 in stage 1 to 7.2 in stage 4.

(a)



(b)



(c)

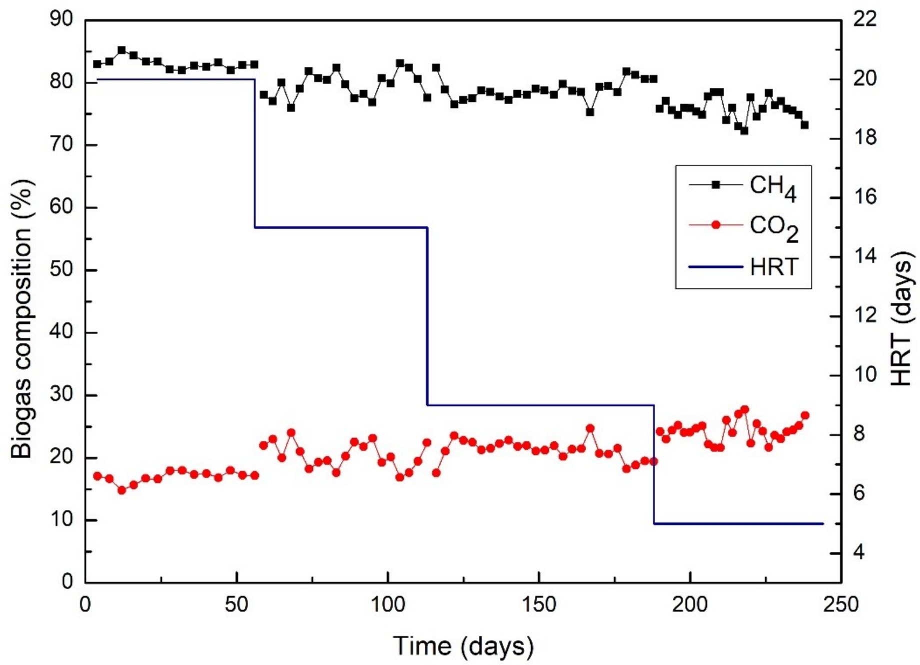


Fig. 5. (a) Methane yield, (b) specific biogas production rate and (c) biogas content at different HRTs in the carbon fiber amended anaerobic biofilm reactor.

Table 7. Performance comparison of anaerobic blackwater digestion among this study and various previous researches.

Parameters	This study				UASB (De Graaff et al., 2010)	UASB (Tervahauta et al., 2014)	UASB (Gallagher & Sharvelle, 2010)	UASB (Gallagher & Sharvelle, 2011)	UASB-ST (Kujawa-Roeleveld et al., 2006)	CSTR (Wendland et al., 2007)	MEC-ST (Zamalloa et al., 2013)
Temperature (℃)	20				25	25	28	28	25	37	30
Reactor volume (L)	0.33				50	50	95	114	200	10	24.2
Average influent TCOD concentration (g/L)	15.2				7.7-9.8	7.1	0.9	0.932	9.5-12.3	8.7	15.5
HRT (d)	20	15	9	5	8.7	9.3	2.3-3.6	2.6-4.0	27-29	20	20-40
OLR (g COD/L-d)	0.77	1.03	1.68	3.01	1.0	0.9	0.23-0.45	0.21-0.39	0.33-0.42	0.44	0.49
TCOD removal (%)	80	74	53	41	78	90	72	72	78	61	85
Specific methane production rate (mL/L-d)	105	163	211	305	200	180	37	31	73	-	50
Methane yield (L/kg COD_{input})	126.8	144	127.5	101.6	211.8	210	137	105.9	170.7	240	113.2
Methane content in biogas (%)	83.0	79.6	78.8	75.5	78	67	62	61.8	66	76	68-77

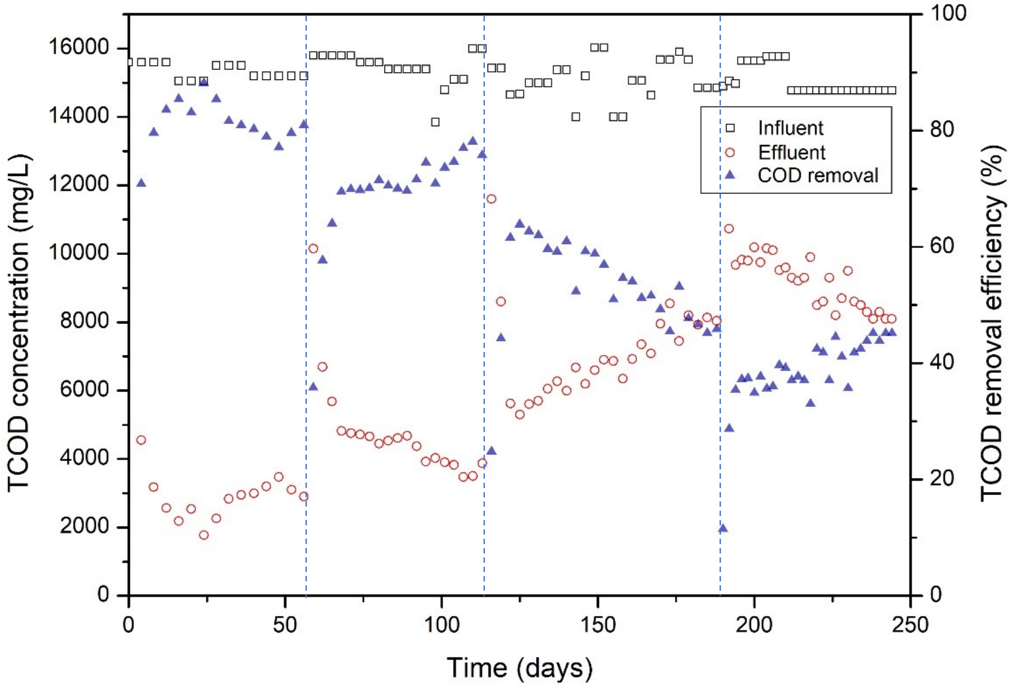
4.3 Organics removal in carbon fiber amended anaerobic biofilm reactor

Organics removal efficiency which is one of the major parameters to evaluate the performance of an anaerobic bioreactor significantly depends on the operation conditions, such as temperatures and HRT (Daud et al., 2018). At an HRT of 20 days, the TCOD removal was 80%, which was an high value compared to that of conventional blackwater digestion systems, such as 61% for CSTR (Wendland et al., 2007), 70% for UASB-ST (Luostarinen & Rintala, 2007), 72% for UASB (Gallagher & Sharvelle, 2011; Gallagher & Sharvelle, 2010). However, with the decrease of the HRT and the increase of OLR, the reactor was stabilized with lower TCOD removal efficiency of 74%, 52%, 41% at HRT of 15, 9, 5 days (Fig. 6a), respectively.

A similar trend in changes in the TSS and VSS reduction as TCOD removal was observed (Fig. 6b). Summarizing the steady state results of each stage, the highest average TSS and VSS removal efficiency of 83% and 81% was achieved respectively, at an HRT of 20 days and an OLR of 0.77 g COD/L-d, and then gradually decreased to 51% and 50% as OLR increased to 3.01 g COD/L-d. The TSS and VSS removal efficiency at 15-days HRT did not obviously differ with that at 20-days HRT due to the limited readily biodegradable organic solids in blackwater. Thus, a 15-days HRT would be recommended in terms of particulate organics or solids removal.

The main component of blackwater is particulate organic content which mainly consists of proteins and carbohydrates (Gallagher & Sharvelle, 2010; Rose et al., 2015), while hydrolysis is commonly considered as the major rate-limiting step of anaerobic digestion of complex organics like blackwater (Christensen, 2011). Some extracellular hydrolytic enzymes produced by hydrolytic and acidogenic bacteria function as one of the key participators in decomposition of complex polymers (Luo et al., 2012; Sträuber et al., 2012). However, hydrolysis products such as various fatty acids and amino acids could inhibit the hydrolytic enzymes production, leading to a reduced hydrolysis rate (Corazza et al., 2005; Song et al., 2016). As discussed later, at high OLR in this study, SCOD in the effluent significantly increased to about 3467 mg/L, which could possibly inhibit the hydrolysis of TSS and VSS.

(a)



(b)

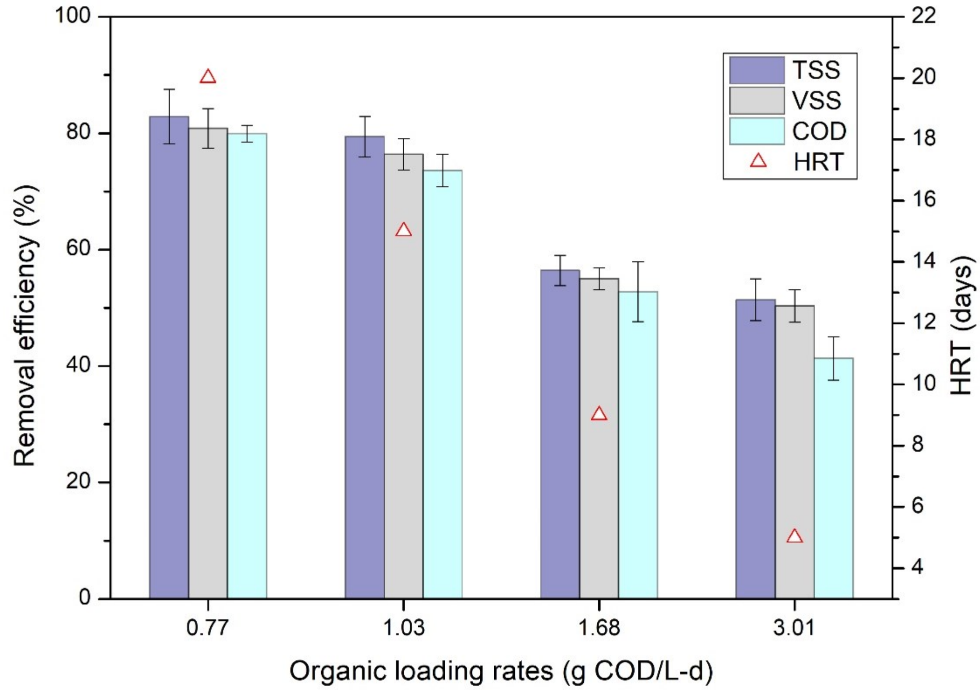


Fig. 6. (a) TCOD concentration in the influent and effluent, and TCOD removal efficiency during the entire operation period; (b) TCOD, TSS and VSS removal efficiency at steady state of each stage.

4.4 Process state variables (pH, TAN, SCOD and SCVFAs)

In an anaerobic digester, pH level in the liquid phase and buffer capacity against pH fluctuation can be influenced by some state variables including carbon dioxide in biogas, alkalinity, the acid concentration, etc., and operational conditions including temperature, HRT, OLR, substrate characteristics and so on (Demitry & McFarland, 2015; Song et al., 2016). The optimum pH range for anaerobic digestion has been proved to be 6.8-7.2 (Cioabla et al., 2012). As shown in Fig. 7, after start-up at an HRT of 20 days with an OLR of 0.77 g COD/L-d, the pH level was maintained at around 7.5-7.8, which was most close to the pH value (8.6) of the inflow blackwater. As a response to the increased OLR of 1.03 g COD/L-d, the pH level dropped immediately to 7.3 and then stabilized at 7.3-7.5. The pH value kept decreasing with the shortened HRTs in the following stages, and finally reached the lowest average pH value of 7.2 in stage 4 with OLR of 3.01 g COD/L-d, which indicates the accumulation of organic acids in the system. The results were also in accordance with the continuously increased carbon dioxide content in the biogas, as the solubility of carbon dioxide decreased with the pH drop (Feng et al., 2018a).

Due to the hydrolysis and decomposition of proteins and other particulate organic content, ammonia could be released during the blackwater digestion (Florentino et al., 2019; Gao et al., 2018a), leading to a higher TAN concentration in the treated effluent than that of influent (~1000 mg/L). As shown in Fig. 8, the average TAN concentration in stage 1 was about 1300 mg/L, while in stage 2, it increased to around 1500 mg/L. For the following two stages with shorter HRTs, the TAN concentration ranged from 1150 to 1300 mg/L. The high TAN concentration in stage 2 with 15-days HRT could be possibly explained by the higher hydrolysis degree with more ammonia released, which could be demonstrated by the highest methane yield in stage 2. However, it is widely accepted that high free ammonia (FA) concentration is more inhibitory to the AD process than the ammonium ion itself, and the inhibition by FA influences mostly only methanogenesis process (Rajagopal et al., 2013; Yenigün & Demirel, 2013). According to the TAN concentration and corresponding pH value, the average FA concentration for each stage was estimated to be 24.5, 17.9, 11.1, 9.4 mg/L, respectively, which is much lower than the inhibitory range (Chen et al., 2008; Rajagopal et al., 2013; Yenigün & Demirel, 2013).

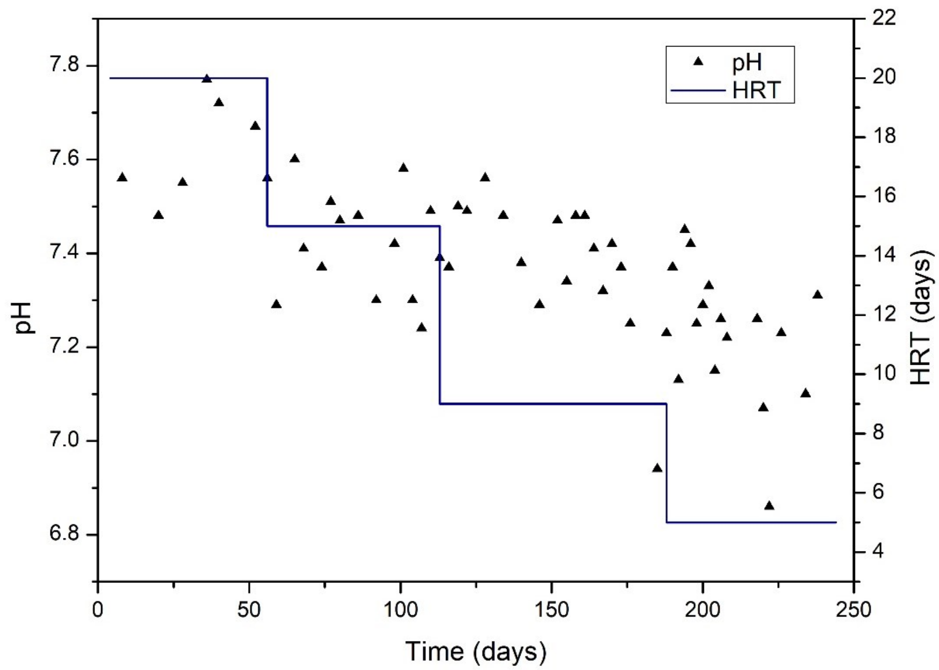


Fig. 7. pH changes during the entire operation period at different HRTs.

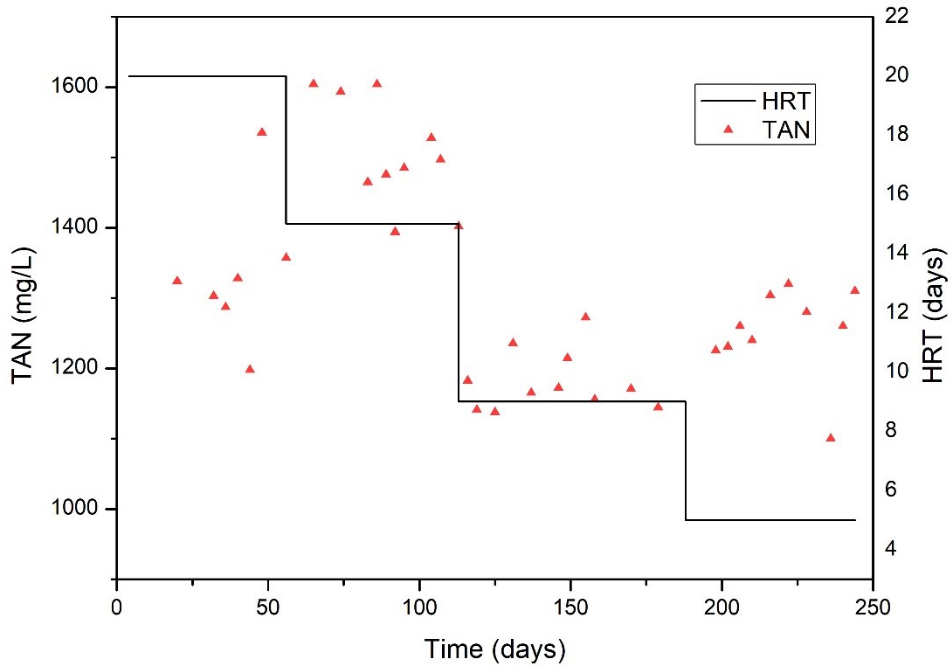


Fig. 8. TAN changes during the entire operation period at different HRTs.

In the anaerobic digestion process, particulate (i.e., insoluble) organic matters are hydrolyzed and converted into long-chain fatty acids, sugars, and amino acids by hydrolytic bacteria (Mata-Alvarez et al., 2000). Then the acidogenic bacteria work on further converting the hydrolysis products into short-chain volatile fatty acids (SCVFAs), such as acetic, propionic, and butyric acids (Gunaseelan, 1997). The main content of SCOD in an anaerobic digester is the monomers hydrolyzed from complex polymers and the SCVFAs acidified from the monomers (Feng et al., 2018a). Excess organic loading rate or wide variations of the inflow characteristics can cause the imbalance between the SCVFA production from the feedstock and the conversion to methane, resulting in SCVFA accumulation, which could further lead to rapid pH drop and finally cause inferior biomethane recovery and process instability (Ahring et al., 1995; Duan et al., 2012; Madsen et al., 2011).

Thus, the concentration of SCVFAs is one of the major indicators informing the system stability and develop potential. At an HRT of 20 and 15 days, as shown in Fig. 9, the total SCVFA levels were as low as 250 mg COD/L, which was 17.4% and 13.5% of the SCOD, respectively, compared with the results (15-24 %) of a previous study for blackwater digestion (De Graaff et al., 2010). However, the total SCVFA concentration kept increasing from stage 3 and accumulated to 1250 mg COD/L at steady state of stage 4, and the percentage of SCVFAs contained in SCOD was increased to 36.2% and 41.6% for stage 3 and 4, respectively. Meanwhile, the SCOD concentration showed a similar stepwise increasing trend as SCVFAs, from 1250 mg/L to above 3500 mg/L. Previous researches suggested the optimum ratio of total SCVFAs to SCOD for sewage sludge digestion was 65.3-79% (Feng et al., 2018a; Feng et al., 2016), while lower ratio of 47% indicated the acidogenesis process was the rate-limiting step over the entire process (Feng et al., 2018a). However, in this study, based on the SCVFA concentration results (Fig. 10) which showed much higher acetate concentration (up to 1000 mg/L) than that of propionate (< 200 mg/L) and butyrate (< 100 mg/L) in the last stage, the methanogenesis process was considered as the rate-limiting step at high OLR of 3.01 g COD/L-d. As the pH (7.2) in this stage was still in the optimum range (6.8-7.2) (Cioabla et al., 2012), stable performance was sustained without system failure and instability. For stage 1 and 2, at lower OLR of 0.75 to 1.03 g COD/L-d when all the SCVFAs stabilized at a low concentration (< 200 mg/L), the AD process in the reactor might be limited by either hydrolysis or acidogenesis.

In summary, the aforementioned results show that the pH, TAN, SCVFAs, and SCOD in the carbon fiber amended anaerobic biofilm reactor were in the normal range with a stable performance during the entire operation at HRTs of 20 to 5 days and OLRs of 0.77 to 3.01 g COD/L-d. Although rate-limiting factors exist, the reactor was proved to have a great capacity to withstand the high organic loading rates for anaerobic blackwater treatment.

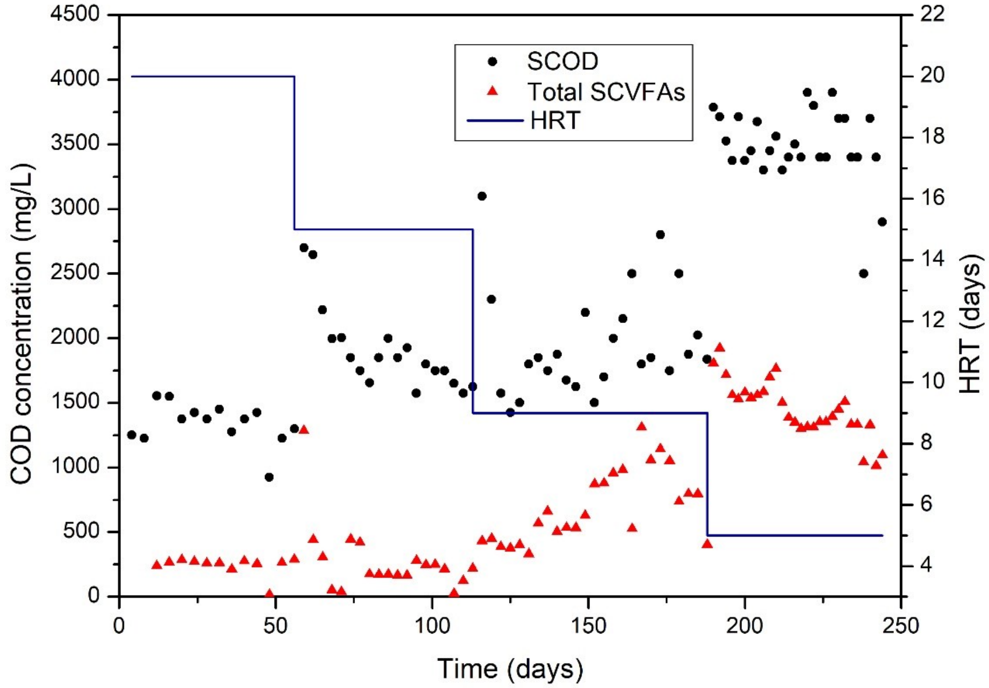


Fig. 9. Changes in SCOD and total SCVFAs (displayed as COD) concentrations during the entire operation period at different HRTs.

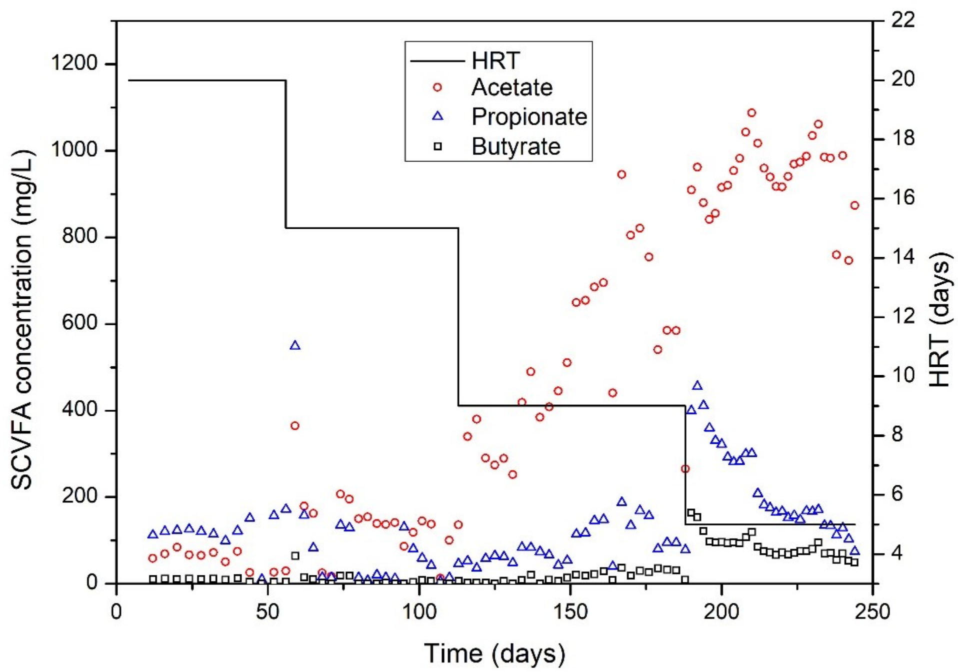


Fig. 10. Changes in SCVFA concentrations during the entire operation period at different HRTs.

Chapter 5

Conclusions and Recommendations

5.1 Conclusions

A list of the major findings from this study is summarized below:

- The performance of carbon fiber amended anaerobic biofilm reactor varied depending on different HRTs. Highest specific methane production rate (304.6 mL/L-d) was achieved at the shortest HRT of 5 days, while the methane content in biogas kept decreasing with the decrease of HRT. Even so, higher purity of methane (75.5-83.0%) was obtained over the entire operation period compared to previous studies on anaerobic treatment of blackwater.
- The maximum methane yield was achieved at HRT of 15 days, which was 38.4% out of 45%. During the first two stages at HRT of 20 and 15 days, the organic matter (TCOD, TSS, VSS) removal was comparable, while the increased OLR led to a deterioration in the removal efficiencies. The results imply that a longer HRT would be desirable for a more stable and higher organic matter removal. However, a shorter HRT with a higher OLR is ideal for higher methane production rate.
- The carbon fiber amended anaerobic biofilm reactor could provide an efficient blackwater treatment performance in terms of specific methane production rate, methane content, organic matter removal, and system stability at ambient temperature. Notably, a high OLR of 3.01 g COD/L-d was achieved in this reactor without any process instability, compared to mostly used OLRs of 0.3-0.5 g COD/L-d in previous studies. Thus, a large amount of heating cost could be saved.

Also, no free ammonia inhibition was observed during the entire operating period.

5.2 Recommendations

The application of conductive materials like carbon fiber appears as a promising technology to improve the methane production, however, the knowledge on this research topic is still limited. The recommendations for future work are as follows:

- Understanding the microbial community structure related to methane production enhancement from blackwater digestion is a key scientific question for the potential application of conductive materials like carbon fiber in digestion efficiency improvement. Thus, microbial community characterization is recommended to get an insight into methanogenesis pathways.
- Exploration on how to further enhance the performance of the carbon fiber amended anaerobic biofilm reactor is required:
  (i) Since the retained biomass and suspended solids on the carbon fiber could contribute to enhanced digestion efficiency in terms of organic removal and methane production. The specific surface areas of the carbon fiber can be increased to provide more conductive area for DIET as well as promoting biofilm formation and suspended solid removal.
  (ii) Microbial electrolysis cells (MECs) offer a novel and transformative solution for enhancement of methane production by bioelectrochemical reactions, with conductive carbon fiber as one of the mostly used material for electrode construction. Therefore, feasibility of incorporating microbial electrolysis into the carbon fiber amended anaerobic biofilm reactor is an

interesting and promising approach to further enhance the performance.

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