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Invited Article

Overview of key operation factors and strategies for improving fermentative volatile fatty acid production and product regulation from sewage sludge

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ABSTRACT

In recent years, volatile fatty acid (VFA) production through anaerobic fermentation of sewage sludge, instead of methane production, has been regarded as a high-value and promising roadmap for sludge stabilization and resource recovery. This review first presents the effects of some essential factors that influence VFA production and composition. In the second part, we present an extensive analysis of conventional pretreatment and co-fermentation strategies ultimately addressed to improving VFA production and composition. Also, the effectiveness of these approaches is summarized in terms of sludge degradation, hydrolysis rate, and VFA production and composition. According to published studies, it is concluded that some pretreatments such as alkaline and thermal pretreatment are the most effective ways to enhance VFA production from sewage sludge. The possible reasons for the improvement of VFA production by different methods are also discussed. Finally, this review also highlights several current technical challenges and opportunities in VFA production with spectrum control, and further related research is proposed.

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Contents

Introduction

In the early 20th century, the activated sludge process began to be applied in wastewater treatment, and now it is one of the most widely used biological technologies around the world. One of its drawbacks is that a large amount of excess sludge is generated and requires proper treatment before final disposal (Appels et al., 2013). The sewage sludge production represents approximately 0.5%–2.0% (volume) of the influent treated with the conventional activated sludge process in wastewater treatment plants (WWTPs) (Montanes et al., 2013). With more and more strict wastewater treatment and management standards, the rapid increase in sewage sludge production is inevitable.

By the end of 2010, sewage sludge production in Europe reached more than 10.0 million tons/year (dry solid), and it is estimated that it will reach 13.0 million tons/year in 2020 (Kelessidis and Stasinakis, 2012). A similar trend was observed in North America: sewage sludge production in the United State increased from 6.9 to above 8.0 million tons/year from 2005 to 2015 (Westerhoff et al., 2015), and Canada produces around 4.0 million tons/year (Foladori et al., 2010). The approximately 3300 existing WWTPs in China produced more than 35 million tons of dry sludge in 2013. It is estimated that sludge production in China will reach 60 million tons/year in 2020 because of stricter discharge standards and more newly built sewage treatment plants (Yang et al., 2015).

Sewage sludge is mainly formed from microorganism growth and particles from influent (Fig. 1) (Nielsen et al., 2012). Among these, the flocs are characterized by the following groups of components: (1) organic carbon compounds (approximately 60% on a dry basis), in large part from biological origin, among which are microorganisms, fiber and extracellular polymeric substances (EPS), (2) inorganic particles, such as silicates and heavy metals, (3) pathogens and other microbiological pollutants, and (4) water, normally varying from 63% to more than 99% (Christensen et al., 2015; Rulkens, 2007).

More specifically, most studies have described the characterization of sewage sludge in terms of protein, carbohydrate, cell biomass, humic acid and DNA. Because of the different determination methods and influent sources used, sludge composition varies in the range of 10%–24% bacterial biomass; 7%–19% carbohydrates; 25%–62% proteins; 8%–29% humic substances; and <3.5% DNA (Values as % volatile solids, VS) (Gonzalez et al., 2018). Except for humic substances, other substrates are biodegradable anaerobically. However, sewage sludge in its combined organized structure is difficultly biodegradable compared to its individual components.

The disposal cost of sewage sludge usually accounts for around 50% of the total operational cost in WWTPs (Appels et al., 2008). With the vast amount of the generated sludge from WWTPs, which also contains a substantial quantity of organic matter, therefore, much attention has been focused on the reuse and recycling of the valuable components from sludge, such as value-added products and bioenergy. Currently, anaerobic digestion has been widely applied in practice for sludge treatment and resource recovery. In place of traditional treatments for methane production, anaerobic

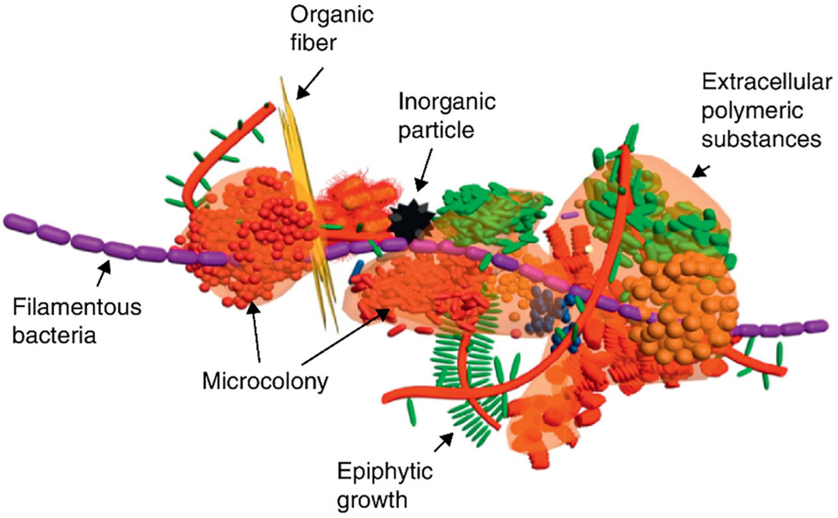

Fig. 1 – Schematic picture of activated sludge adapted from (Nielsen et al., 2012).

fermentation for VFA production is a promising technology in sludge treatment and resource recovery (Fang et al., 2018). As shown in Table 1, VFAs are short-chain fatty acids that consist of six or fewer carbon atoms.

Compared to methane, VFAs have higher value and a wider range of applications, such as in the food and pharmaceutical industries as preservatives (Lee et al., 2014), in the production of esters, bioplastics (Kleerebezem and van Loosdrecht, 2007) and bioenergy (Uyar et al., 2009), as well as in the biological removal of nutrients from wastewater as a carbon source (Agler et al., 2011; Henze, 1991; Li et al., 2011). Therefore, more and more studies have shifted to VFA production through the fermentation of organic wastes. However, VFA production from sewage sludge is usually limited by low hydrolysis rates and biodegradation. To overcome these problems, different sludge-treatment parameters have been thoroughly evaluated at various scales, including bench, pilot, and large-scale. Lee et al. (2014) reviewed the production and application of VFA derived from various wastes and also discussed the factors influencing the VFA yields from the wastes. However, most published studies have focused on improving VFA yields, while fewer paid attention to investigating how to control the product spectrum in reactors under different operating conditions. In the present work, a glance was first taken at the main characteristics of sewage sludge. Following this, a discussion of the effects of the different operational parameters considered to influence the VFA yield and composition is presented. In line with the discussion of the results, various approaches (including pretreatment and co-fermentation) are comprehensively examined. Furthermore, the underlying mechanisms that influence the hydrolysis rate, VFA production and composition are identified. Finally, the possible technical issues and present research gaps for each method are discussed and summarized.

Table 1 – Names, formulas and chemical structures of volatile fatty acids.

Compound	Formula	Chemical structure
Acetic acid	$C_2H_4O_2$	CH_3-COOH
Propionic acid	$C_3H_6O_2$	CH_3-CH_2-COOH
n-butyric acid	$C_4H_8O_2$	CH_3-$(CH_2)_2$-COOH
iso-butyric acid	$C_4H_8O_2$	$(CH_3)_2$-CH-COOH
n-valeric acid	$C_5H_{10}O_2$	CH_3-$(CH_2)_3$-COOH
iso-valeric acid	$C_5H_{10}O_2$	$(CH_3)_2$-$CHCH_2$-COOH
n-caproic acid	$C_6H_{12}O_2$	CH_3-$(CH_2)_4$-COOH

1. Key factors influencing VFA production from sewage sludge

As illustrated in Fig. 2, during anaerobic fermentation of sewage sludge, the complex organic matters, such as proteins, carbohydrates, and lipids, are firstly converted to their soluble forms, which are then rapidly fermented to pyruvate through glycolysis, and finally to organic acids. Since anaerobic fermentation is a multi-stage process with chemical and biological reactions, different operational conditions such as pH, temperature, C/N ratio and solids retention time (SRT), as well as organic loading rate (OLR), have great effects on the performance of the process, thus influencing the yield and the distribution of VFA (Cokgor et al., 2009; Rughoonundun et al., 2012; Wijekoon et al., 2011; Yuan et al., 2011). Most of the researchers examined one condition at a time and there are only a few studies evaluating their interactive effects. In view of that, the factors affecting the VFA yields from sewage sludge are discussed individually as follows.

1.1. *pH*

pH is one of the most important factors that significantly influence the efficiency of hydrolysis and acidification (Horiuchi et al., 2002; Lin and Li, 2018). Even though anaerobic bacteria have a wide adaption ability, most of the acidogens cannot survive under extremely acidic ($pH \leq 3$) or alkaline ($pH \geq 12$) conditions (Biswas et al., 2009). As shown in Table 2,

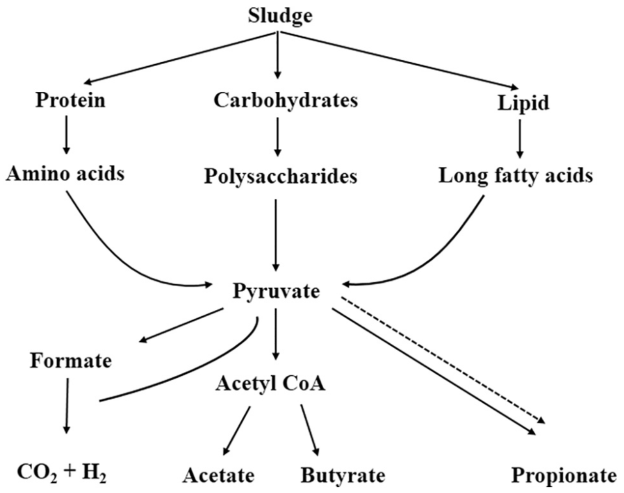

Fig. 2 – Major pathways of anaerobic fermentation of sewage sludge.

various studies concluded that the optimal pH for the maximum VFA yield from sludge is in the range of 8–11 (Azman et al., 2015; Liu et al., 2012). The acidogenesis process showed poor performance under alkaline conditions compared to neutral pH (Ma et al., 2016). Hence, the increased VFA production at alkaline pH was probably contributed by the increased hydrolysis rate and biodegradation due to disintegration and solubilization of protein and carbohydrate from bacterial cells. Meanwhile, proteins usually are more biodegradable substrates than carbohydrates (Wu et al., 2009; Yuan et al., 2006). This effect is similar to that of alkaline pretreatment of the sludge, as discussed in Section 2.2.1. In addition, alkaline conditions inhibit the activity of methanogenesis, which can consume produced VFA for methane formation (Jiang et al., 2013; Jie et al., 2014).

pH also influences the fractions of individual VFA, especially regarding acetate, propionate and butyrate (Feng et al., 2009a). At low pH from 4.0 to 5.0, the production of ethanol increases, while at higher pH more VFA are produced (Ren et al., 1997). The level of butyrate is usually high and is the dominant VFA species at pH values lower than 5.5, while acetate is produced as the major product at neutral and alkaline pH, and information regarding propionate dominance is seldom reported (Cokgor et al., 2009; Temudo et al., 2007). It is necessary to further investigate the influence of pH on specific microbial communities, which directly dominate the VFA composition.

1.2. *Temperature*

Temperature is another important factor influencing VFA production from anaerobic fermentation. Generally, anaerobic fermentation for VFA production from sewage sludge has been carried out in a wide range of temperatures from psychrophilic (<20°C), mesophilic (25–40°C) to thermophilic (45–60°C) conditions (Ferreiro and Soto, 2003; Feng et al., 2019; Kashyap et al., 2003; Zhuo et al., 2012). A brief literature review is given in Table 3. Many research studies indicated that a proper increase in fermentation temperature could lead to enhancement in VFA production by 20% to more than 2-fold (Skalsky and Daigger, 1995; Yuan et al., 2016). This positive result was mainly attributed to the presence of a greater amount of soluble proteins and carbohydrates due to higher activities of enzymes, such as protease and α-glucosidase (Moser-Engeler et al., 1998). Moreover, Yuan et al. (2011) stated that the hydrolysis rate increased by over 50% when the fermentation temperature of sludge increased from 14.6 to 24°C. From a thermodynamics point of view, the higher temperature reduces the initial Gibbs energy in the biodegradation of organic matter, leading to a faster digestion rate (Kanokwan, 2006).

However, the influence of temperature on the VFA profile from the fermentation process is limited and contradictory. Ahn and Speece (2006) found that acetate was dominant either in mesophilic or thermophilic fermentation from primary sludge (PS). Yuan et al. (2011) performed the fermentation of waste activated sludge (WAS) from 4.0 to 24.6°C. As the temperature increased from 4.0 to 14.0°C, the percentage of propionate and butyrate increased slightly from 20% to 29% and from 11% to 16%, respectively; while the percentage of acetate declined from 55% to 43%. Conversely, Yuan et al. (2016) demonstrated that mesophilic fermentation of WAS at (30 ± 2)°C under alkaline conditions presented higher acetate production as the major component of VFA compared to psychrophilic conditions at (15 ± 2)°C.

Even though several advantages were reported for operation under thermophilic conditions, some disadvantages are worth considering. Firstly, the anaerobic microorganisms in

Table 2 – Effect of pH on volatile fatty acid (VFA) production and composition.

Type of wastes	pH range studied	Optimal pH	VFA yield	Main VFA compositions at maximal yield	Reference
Waste activated sludge	4–11	10	196 mg/g VSS	Acetic, propionic and iso-valeric acids	Chen et al. (2006)
Waste activated sludge	5–12	10	302 mg COD/g VSS	Acetic and propionic acids	Jie et al. (2014)
Waste activated sludge	6–9	8	520 mg COD/g VSS	Propionic acid	Feng et al. (2009a)
Proteinaceous sewage sludge	3–11	9	600 mg COD/g VS	Acetic and propionic acids	Liu et al. (2012)
Primary sludge	3–11	10	302 mg COD/g VSS	Acetic, propionic and iso-valeric acids	Wu et al. (2009)

Table 3 – Effect of temperature on volatile fatty acid (VFA) production and composition.

Type of wastes	Temperature range studied	Optimal temperature	VFA yield	Main VFA compositions under maximal yield	Reference
Primary sludge	10–35°C	35°C	340 mg COD/g VSS	Acetic and propionic acids	Ferreiro and Soto (2003)
Waste activated sludge	40–60°C	50°C	240 mg/g VSS	Acetic and butyric acids	Xiong et al. (2012)
Waste activated sludge	10–35°C	35°C	192 mg COD/g VSS	Acetic acid	Feng et al. (2009a)
Waste activated sludge	10–55°C	37°C	197 mg COD/g VS	Acetic acid	Zhuo et al. (2012)
Waste activated sludge	4–24.6°C	24.6°C	About 420 mg/g VSS	Acetic and butyric acids	Yuan et al. (2011)

thermophilic conditions are more sensitive to environmental changes than in a mesophilic process (Kim et al., 2006). It was reported that thermophilic process failure could occur if the rate of temperature change exceeded 1.0°C/day, and thus the changes in temperature should be less than 0.6°C/day to maintain stable conditions for VFA production (Perot et al., 1988). Besides, higher temperature increases the pKa of VFA, which further increases undissociated fractions and raises the toxic risk to bacteria, especially at low pH and high VFA concentrations.

1.3. *C/N ratio*

The performance of anaerobic fermentation is also significantly affected by the carbon-to-nitrogen (C/N) ratio through influencing the microbial metabolism. An optimum C/N ratio is required for anaerobic fermentation because an appropriate nutrient balance is essential to anaerobic bacteria for their growth and maintenance under a stable environment (Guerrero et al., 1999; Yu and Fang, 2001). It was reported that C/N ratios ranging from 15 to 70 (on a mass basis) have been most commonly used for anaerobic digestion (Kayhanian and Tchobanoglous, 1992). Generally, a C/N ratio range of 20–30 is considered to be the optimum condition for anaerobic fermentation (Zhang et al., 2014). However, sludge itself is a substrate with a high nitrogen content but low amount of biodegradable carbon, so its C/N ratio is usually in the range between 6 and 9. Therefore, a common method used to enhance efficiency is by sludge co-fermentation with carbon-rich substrates (Rughoonundun et al., 2012). Chen et al. (2013b) studied the performance of VFA production by co-fermentation of WAS and kitchen waste of different C/N ratios, which concluded that the conditions for optimum VFA production were found at a C/N ratio of 22 under alkaline conditions, through significantly enhancing the general activity of anaerobic microorganisms as well as the activities of key acid-producing enzymes. However, in recent studies it was found that the fermentation processes performed well even at low C/N ratio (10–20). Liu et al. (2008) concluded that the maximum total VFA yield of 152.1 mg TCOD/g VS_{added} was obtained from sludge mixtures with a high initial C/N ratio of 15.1 compared to ratios of 12.2 and 5.0. The above findings indicate that the C/N ratio plays an important role in the anaerobic fermentation process of sewage sludge. Thus, a balanced and proper C/N ratio in the feedstock is also a crucial factor to boost the acidogenic activity.

Although it has been reported that when the C/N ratio increased from 5 to 30, the butyrate fraction gradually increased as the acetate fraction declined (Liu et al., 2008), it is however difficult to conclude that there is direct relationship between C/N ratio and the distribution of individual VFAs like that for pH. Thus, the operating parameters and characteristics of mixed substrates other than C/N ratio may play more important roles. Further studies should pursued in the future.

1.4. *Retention time*

The solid retention time (SRT) and hydraulic retention time (HRT) influence waste hydrolysis and VFA production during anaerobic fermentation (Jankowska et al., 2018). The SRT is equal to HRT in most cases of VFA production from sewage sludge, when the solid substrate and microbial culture are mixed during continuous operation. Selecting proper HRT/SRT can avoid wash-out of slow-growing bacteria (Ghosh and Pohland, 1974).

While more than 20 days anaerobic digestion are required for methane production, various research studies proposed that a lower SRT between 5 and 10 days is beneficial to VFA production from sewage sludge, because a relatively shorter SRT can prevent the growth of methanogens. However, in order to enhance the hydrolysis rate of the sludge, the retention time should be optimized (Ferrer et al., 2010; Miron et al., 2000; Xiong et al., 2012). Miron et al. (2000) examined the effect of SRT between 3 and 15 days on the hydrolysis, acidification and methanogenesis of PS. The results indicated that acidogenic conditions prevailed when the SRT was lower than 8 days, while the methanogenic process prevailed when the SRT was longer than 10 days (Table 4). Similar results were published by Yuan et al. (2009). Some authors suggested that the SRT of PS for VFA production is 5–6 days, which is shorter than that of WAS, because the WAS is more difficult to solubilize due to its high fraction of cell biomass (Bouzas et al., 2002; Jiang et al., 2007).

Unfortunately, no consistent conclusion has been reached regarding the changes in VFA distribution from anaerobic sludge fermentation with retention time. Yuan et al. (2009) stated that the dominant product shifted from acetate to higher amounts of propionate, butyrate and caproate as the SRT increased from 6 to 10 days. In contrast, another study showed that with the increase in SRT from 4 to 16 days, the fraction of acetate increased and the percent of propionate

Table 4 – Effect of SRT on volatile fatty acid (VFA) production and composition.

Type of waste	SRT range studied	Optimal SRT	VFA yield	Main VFA compositions under maximal yield	Reference
Waste activated sludge	0–312 hr	5 days	240 mg/g VSS	Acetic and butyric acids	Xiong et al. (2012)
Waste activated sludge	5–10 days	10 days	140 mg/g TCOD	Acetic acid	Yuan et al. (2009)
Waste activated sludge	4–16 days	12 days	192 mg COD/g VSS	Acetic acid	Feng et al. (2009b)
Primary sludge	4–8 days	6 days	36 mg HAc/L	Acetic acid	Bouzas et al. (2002)
Primary sludge	4–10 days	6 days	214 mg/g VSS	Acetic and butyric acids	Bouzas et al. (2002)
Primary sludge + waste activated sludge	About 10–30 days	9.4 days	391 mg/g VSS	Propionic acid	Ferrer et al. (2010)

decreased (Feng et al., 2009b). Probably because of different operating conditions, a confusing view was obtained when the SRT or HRT was considered as a single parameter. To further understand the underlying mechanisms behind these observations, it would be interesting to examine the microbial community in the anaerobic fermentation process under different SRTs.

1.5. *Organic loading rate*

The organic loading rate (OLR), which is calculated from the substrate concentration and hydraulic retention time, indicates how many kilograms of organic substrates are loaded daily per unit of digester volume (Wijekoon et al., 2011; Liu et al., 2019). So far, most OLRs in anaerobic fermentation of waste sludge are no more than 30 g TS/(L·day), whether in continuous or batch systems (Banerjee et al., 1999; Chen et al., 2006). On the premise of system stability, a high OLR means high sludge treatment capacity and high VFA production, hence the yield or production of VFA increases with increasing OLR within a reasonable range (Min et al., 2005). However, at a higher OLR achieved through decreasing HRT, lower VFA production could be observed, temporarily or permanently, due to lower hydrolysis efficiency. For example, Banerjee et al. (1999) demonstrated that the total VFA production from PS fermentation decreased from 0.4 to 0.3 g/L, when the OLR increased from 4 to 7 g TS/(L·day) by decreasing HRT from 30 to 18 hr.

The OLR also significantly influences the VFA distribution, especially when the OLR is high either from an increase in the feed concentration or decrease in HRT, commonly with a shift from more-oxidized compounds to more-reduced compounds, such as propionate production derived from the lactate-propionate pathway (Dijkstra, 1994; Li et al., 2016; Rodríguez et al., 2006). Yu et al. (2002) reported that under mesophilic conditions, when the OLR increased from 4 to 24 kg COD/(m^3·day), the percentage of propionate increased from 13% to 41% of the total VFA, while the percentage of acetate declined from 40% to 17%. Similar results were observed during VFA production from food waste and starchy wastewater (Yu, 2001). However, if the OLR increases further beyond a certain limit, the fermentation system could collapse due to lactate accumulation and pH drop (Jouany, 2006). It is necessary to determine and clarify the optimal range of OLR with different bioreactors.

1.6. *Trace elements*

Besides the carbon source and the nutrients nitrogen and phosphorus, trace elements are also necessary for anaerobic microorganism metabolism. As shown in Table 5, these trace elements (e.g. cobalt, nickel, zinc and iron) play a very important role in activating and maintaining enzymatic activities in the anaerobic fermentation process (Karlsson et al., 2012; Thanh et al., 2016). For example, Co is a cofactor of carbon monoxide dehydrogenase (CODH), which is a key enzyme both for the production and consumption of acetate (Roth et al., 1996; Zandvoort et al., 2006a). Similarly, Ni and Fe are essential cofactors of CODH. Moreover, they are also essential for the activity of many other hydrogenases, and thus affect both fermentative and methanogenic microorganisms (Vignais and Billoud, 2007). Besides, molybdenum (Mo) and tungsten (W) are related to formate dehydrogenases, which catalyze formate production by propionate oxidizers (Dong et al., 1994).

However, most researchers focused on trace elements with the objective of improving biogas production through VFA utilization (Zandvoort et al., 2006b; Zitomer et al., 2008; Yazdanpanah et al., 2018), and there is very limited information on the role of trace elements in VFA production. Lin et al. (1998) systematically investigated the effect of trace element supplementation on the anaerobic degradation of butyrate. Kim et al. (2002) reported that the addition of Ca, Fe, Co, and Ni accelerated propionate production at high concentrations in thermophilic anaerobic digestion. Furthermore, Karlsson et al. (2012) investigated the effect of dosing trace elements (Fe, Co and Ni) on biogas production and indicated that the addition of trace elements led to an increase in acetate concentration with a concomitant increase in biogas production. In short, few studies specifically focused on the effects of trace elements on VFA yield and also the microbial community in WAS fermentation processes, and further investigations would be required to understand how trace elements enhance VFA yield from sewage sludge.

Table 5 – Selected trace elements in functional enzymes of the anaerobic digestion process.

Nutrient	Enzymes	Reference
Co	Corrinoids, CODH, Proteases	Roth et al. (1996)
Cu	Hydrogenase, CO-dehydrogenase	Zandvoort et al. (2006a)
Fe	CODH, sulphides, Formate dehydrogenase,	Karlsson et al. (2012)
Wo or W	Formylmethanofuran-dehydrogenase, Aldehyde-oxydoreductase	Dong et al. (1994)

2. Typical pretreatment methods for enhancing VFA production from sewage sludge

The hydrolysis of sludge limits the rate and extent of organic degradation in the anaerobic fermentation process, as proteins, carbohydrates and lipids as well as soluble inert materials are wrapped in sludge flocs and microbial cell walls, which thus inhibit the release and degradation of organic matter (Carrere et al., 2010). Therefore, suitable approaches are usually employed to enhance sludge hydrolysis to improve the anaerobic fermentation performance (Ariunbaatar et al., 2014). As shown in Table 6, various pretreatment methods have been explored to enhance the sludge solubilization and further VFA production. These approaches are mainly divided into physical, chemical, and biological processes, as well as their combination (Braguglia et al., 2012; Chu et al., 2002; Ge et al., 2010; Yeom et al., 2002).

2.1. *Physical pretreatments*

2.1.1. *Ultrasonic pretreatment*

Ultrasonic pretreatment generates cavitation that leads to physical and chemical changes in the liquid phase, which in turn produces physical effects (shear forces) at low frequency and even free radicals, especially under high frequency conditions (Carrere et al., 2010). The efficiency of sludge disintegration by ultrasonic pretreatment mainly depends on the operating parameters such as frequency and sludge concentration (Neumann et al., 2017; Zhang et al., 2007). According to Show et al. (2007), the optimal range of solid content for ultrasonic pretreatment should not be higher than 3.2% TS. Solid content above 3.2% TS hinders the formation of cavitation bubbles and reduces the efficiency of pretreatment as a consequence of the increase of viscosity. Generally, ultrasonic pretreatment of sludge is conducted at 20–40 kHz. It is worth noticing that hydroxyl radicals are generated when the frequency is above 150 kHz, as described by Chatel (2017).

Ultrasonic pretreatment leads to sludge floc disintegration and lysis of microorganisms (Zabaneh and Bar, 1991), reduction in particle size and cell damage, which consequently increase the hydrolysis rate (Cella et al., 2016). Recently, several researchers have examined the impact of the ultrasonic pretreatment of sludge on VFA production. According to Yan et al. (2010), the rate and extent of hydrolysis (expressed by soluble protein and carbohydrate concentrations) increased thanks to the ultrasonic pretreatment. Maximum VFA accumulation was reached (3110 mg COD/L) in anaerobic fermentation of sludge under alkaline conditions. After 72 hr of fermentation, the substrate was treated with ultrasound at 1.0 kW/L. This step increased the VFA recovery 2-fold compared with the control group (not treated with ultrasound). Similar results were also observed, where ultrasonic pretreatment resulted in about 4-fold higher enhancement in VFA yield for the pretreated sludge compared to untreated samples (Guo et al., 2014). During the ultrasonic process, the temperature measurably increased along with the increase in operation time, thus it is necessary to consider and distinguish the different effects of temperature on solubilization and biodegradation. In one study regarding methane production from WAS, Le et al. (2016) found that better performance can be achieved by coupling the effects of ultrasonic and thermal pretreatments. When keeping a mild temperature below 35°C, the biodegradability of WAS increased only 2%, although an increase in soluble chemical oxygen demand (SCOD) occurred (Cella et al., 2016). One possible reason for this result could be explained by the contribution of thermal effects. However, little information is available about whether sonication or the thermal effect contribute more to the solubility of proteins and carbohydrates and the increase in biodegradability of sewage sludge.

Information regarding the mechanism by which ultrasonic pretreatment influences VFA composition is limited. In the same study performed by Yan et al. (2010), it was found that acetate was a dominant VFA at different ultrasonic energy densities, but its concentration decreased by approximately 7.4% while odd VFAs (propionate and valerate) increased nearly 10% when the ultrasonic energy density increased from 0 to 4.0 kW/L. The author explained this phenomenon based on variations of related key enzymes, while the remaining mechanisms could merit further investigation.

In general, ultrasonic pretreatment is relatively energy-intensive and requires careful maintenance operations due to irradiation. Combination with other chemical pretreatment methods, such as alkaline and acid treatments, might be a feasible way to elevate the application potential of the ultrasound process. Liu et al. (2009) reported that the total VFA yield from WAS fermentation increased by 68.2% with ultrasonic-alkaline pretreatment. Another drawback of ultrasonic pretreatment, besides being an energy-intensive technique, is that the sludge concentration should be below 4%; otherwise, the efficiency significantly deceases (Pilli et al., 2016).

2.1.2. *Thermal pretreatment*

Thermal pretreatment has been shown to have positive impacts on the disintegration of sludge flocs and cell membranes, resulting in the solubilization of organic compounds, leading further to improved performance within the anaerobic process (Haug et al., 1978; Neyens and Baeyens, 2003). The performance of thermal pretreatment depends on the temperature and operation time, in which the pretreatment temperature is more influential in sludge disintegration than the operation time (Bougrier et al., 2008). Most studies were carried out at mild-thermal temperatures (55–100°C) and high-thermal temperatures (100–220°C) from minutes to

Table 6 – Effect of different pretreatment methods on volatile fatty acid (VFA) yield from sewage sludge.

Pretreatment method	Sludge type	Operating conditions	VFA yield (mg COD/g VSS)	Reference
Ultrasound	Waste activated sludge	Frequency of 20 kHz, energy, density of 1.0 kW/L, 10 min, pH 10.0, 20°C	445	Yan et al. (2010)
Ultrasound	Waste activated sludge	Frequency of 28 kHz, 60 min, pH 12	230	Liu et al. (2009)
Thermal	Waste activated sludge	160°C at 6 bar, 37°C without pH control	0.2[a]	Morgan-Sagastume et al. (2011)
Alkaline	Waste activated sludge	pH 9.0 (NaOH), mesophilic (35±2)°C	298	Zhang et al. (2009)
Alkaline	Waste activated sludge	pH 10.0 (NaOH), 20-22°C	256.2	Yuan et al. (2006)
Alkaline	Waste activated sludge	pH 10.0 ($CaOH_2$), 25°C	215.5	Li et al. (2011)
Free nitrous acid (FNA)	Waste activated sludge	(20±1)°C, 1.54 mg FNA/L, pH 10	370.1	Zhao et al. (2015a)
Enzyme	Waste activated sludge	Mixed enzymes[b], dosage of 0.06 g/g DS, 50°C without pH control	211.7	Luo et al. (2011)
Enzyme	Waste activated sludge	Amylase dosage of 0.1 g/g sludge, 28 hr, 35°C without pH control	93.7	Yu et al. (2013)
Bio-surfactant	Waste activated sludge	Rhamnolipid dosage of 0.05 g/g dry sludge, 30±1°C	311	Huang et al. (2015)
Bio-surfactant	MBR[e] sludge	Alkyl polyglcoside[c], dosage of 0.2 g/g dry sludge, 35±1°C, pH 11.0	282.9	Zhao et al. (2015b)
Alkaline + thermal	Waste activated sludge	90°C, pH 12.0	220	Liu et al. (2009)
Surfactant + biological enzyme	Waste activated sludge	Sodium dodecyl sulfate (SDS) dosage of 0.10 g/g DS + Mixed enzymes 0.06 g/g DS[d] + 50°C without pH control	240.8	Luo et al. (2011)

[a] The unit is gVFA/g TCOD.
[b] Mixed (mixed-enzymes 0.06 g/g DS, protease: a-amylase = 3:1).
[c] Alkyl polyglucoside.
[d] SDS + ME (sodium dodecyl sulfate (SDS) 0.10 g/g dry sludge (DS), mixed-enzyme, 0.06 g/g DS, protease: a-amylase = 3:1.
[e] Membrane bioreactor.

several hours (Chen et al., 2019; Choi et al., 2018; Mottet et al., 2009; Nazari et al., 2017).

Reduction in particle size can be observed at a mild-thermal temperature of 50°C after 20 min (Audrey et al., 2011), and as expected, the deflocculation increased with increased temperature, which improved the hydrolysis rate (Vavilin et al., 2008). However, higher reduction in particle size was observed at 190°C compared to the untreated sample due to the formation of chemical bonds (Bougrier et al., 2006). Besides, another effect of thermal pretreatment on sludge is the increase in solubilization of proteins and carbohydrates (Bougrier et al., 2008; Tsapekos et al., 2016). Seemingly, protein is more prone to being solubilized than carbohydrate during mild-thermal pretreatment. Audrey et al. (2011) demonstrated that the solubilization degree of protein and carbohydrate reached approximately 19% and 7%, respectively, at 95°C after 20 min. The solubilization of organic matter can increase biodegradation, as expected. Nevertheless, the mild-thermal pretreatment only contributed to 20% of protein and carbohydrate degradation from their total fraction.

Most of the studies in recent decades have focused on the use of thermal pretreatment to enhance the dewatering of sludge and methane production (Gavala et al., 2003; Tanaka et al., 1997). Although there have been few investigations on the thermal pretreatment of sewage sludge for VFA production, they have yielded some encouraging results (Wilson et al., 2009). For instance, Wilson et al. (2009) investigated the effect of thermal pretreatment on the anaerobic digestion of sludge rich in lipid and protein, and found that the VFA production was substantially improved by 37% after thermal pretreatment at 170°C was applied. Besides, several researchers combined thermal pretreatment and other methods to obtain more favorable results (Morgan-Sagastume et al., 2011). For example, Morgan-Sagastume et al. (2011) observed considerably increased WAS solubilization with high-pressure thermal pretreatment at 160°C and 6 bar in a full-scale plant. In a sequential fermentation process, this resulted in 2- to 5-fold increase in VFA yield and a 4- to 6-fold increase in VFA production rate. In another study, the authors pretreated substrates with high triglyceride content and observed substantial enhancement in biogas production owing to the contribution of higher VFA concentrations after thermal pretreatment (Hiraoka et al., 1984). In contrast, Dwyer et al. (2008) reported that sludge biodegradability started to decrease at 170–190°C in spite of achieving high solubilization efficiencies. Actually, too high a temperature may lead to the formation of chemical bonds and result in the Maillard reaction, which occurs between carbohydrates and amino acids (Weemaes and Verstraete, 1998). There is still a lack of relevant studies showing the precise effects of refractory substrates on VFA production, and the optimal conditions in which the maximal positive effects from increased solubilization and minimal negative impacts of solubilized refractory compounds at higher temperature can be sustained.

Thus, the results show that the thermal pretreatment of WAS can enhance VFA yields, due to its high efficiency in the solubilization of cell membranes leading to cell lysis and subsequent release of intracellular organic matter. Moreover, elevated temperature in thermal pretreatment processes has the potential to produce Class A biosolids by destroying pathogens. Compared to mild-thermal pretreatment, however, higher temperature perhaps has a negative energy balance and attention should be paid to avoiding refractory solubilization.

2.2. Chemical pretreatments

2.2.1. Alkaline pretreatment

Alkaline pretreatments have been widely applied in anaerobic fermentation of sewage sludge, because the alkaline reagents can largely destroy sludge floc structures, cell walls and cell membranes in 10–24 hr under highly alkaline conditions (pH 8.0–12.5) by the action of hydroxyl ions (Kim et al., 2003; Neyens et al., 2004). VFA production from sludge with alkaline pretreatment is usually combined with pH control under alkaline conditions (Yuan et al., 2006; Zhao et al., 2018). Due to destruction of the amide groups in EPS during alkaline pretreatment (Wingender et al., 1999), damage to EPS was observed at pH 7.00–12.50, and rapid damage occurred at pH 11.50–12.00, which increased its solubilization (Xiao et al., 2015).

Generally, the application of alkaline pretreatment could improve VFA production from sludge in two ways: (1) by increasing the solubilization of EPS and even intracellular organic matter in the sludge and then providing more biodegradable substrates for the acidogenic microorganisms, and (2) concomitant biological processes decreasing and/or preventing the activity of methanogens (Devlin et al., 2011; Dogan and Sanin, 2009). Yuan et al. (2006) investigated the VFA production from sludge fermentation in batch tests at room temperature, which demonstrated that the maximum VFA yield of 256 mg COD/g VSS obtained under alkaline conditions (pH 10.0, NaOH) was 2.7-fold higher than that in the blank group without alkaline addition. Besides, it was also indicated that sludge fermentation under alkaline conditions (pH 10.0, NaOH) significantly decreased methanogenic activity, resulting in lower VFA consumption and lower methane production.

However, too much addition of alkalinity (pH > 12) would generate refractory or toxic compounds that negatively impact on acidogenic bacteria, resulting in a reduction in VFA yields or increase in lag-phase time, although the SCOD continuously increases (Chen et al., 2006). Similarly, Kim et al. (2013) reported that the maximal methane production from anaerobic digestion of sludge was obtained at pH 10.0, and concluded that conversion of excess sludge to soluble matter by NaOH is not always beneficial for anaerobic digestion. Besides, concentrations of Na^+ above 3.5 g/L can cause subsequent inhibition of anaerobic fermentation (Appels et al., 2008; Mouneimne et al., 2003).

The types of reagents used also affect the performance of VFA production from sewage sludge. Due to higher solubilization efficiency and VS removal than other types of alkaline reagents, NaOH has been used as the major alkali reagent (Chen et al., 2006; Yu et al., 2008). However, due to the function of calcium bridging, $Ca(OH)_2$ leads to better dewatering performance for sludge (Su et al., 2013). As discussed in Section 2.1, the dominant VFA composition was acetate under alkaline conditions during the anaerobic fermentation process.

2.2.2. Free nitrous acid (FNA) pretreatment

In recent years, a novel chemical pretreatment method based on free nitrous acid (FNA) in enhancing the anaerobic digestion of sludge has attracted great interest (Lu et al., 2019; Wang et al., 2013b, 2019; Wu et al., 2018). This treatment shows a strong biocidal effect, damaging cell membranes and even killing 50%–80% microorganisms in sludge at ppm (parts per million) levels within 12–48 hr (Pijuan et al., 2012; Wang et al., 2013b). However, FNA could not destroy recalcitrant materials in sludge, such as humic acid, within the dosage range of positive effect on anaerobic digestion. The nitrite ion under acidic conditions could generate several reactive derivatives, as shown in Eq. (1):

$$HNO_2 + HNO_2 \leftrightarrow N_2O_3 + H_2O \leftrightarrow NO_2 + NO + H_2O \tag{1}$$

The produced small molecules, such as dinitrogen trioxide (N_2O_3), nitrogen dioxide (NO_2) and nitric oxide (NO), can easily pass through cell membranes, and cause microbial inactivation (Jiang et al., 2011). Additionally, FNA or its derivatives also break down EPS by oxidation and depolymerization of proteins and carbohydrates (Zhang et al., 2015). The solubilization of EPS and cells resulted in enhancement of the hydrolysis rate and biodegradability of WAS, with the highest improvement of approximately 50% and 27%, respectively (Wang et al., 2013a).

The first work dealing with VFA production from WAS with FNA pretreatment was carried out by Zhao et al. (2015a). They reported that the dosage of 1.54 mg HNO_2-N/L in combination with alkaline fermentation at pH 10.0 increased SCOD disintegration by 193.3%, resulting in 4.7-fold improvement in VFA yields, at 370 mg COD/g VSS. Additionally, the fermentation time decreased from 15 to 4 days when the VFA reached highest concentration. These results were in conformity with another study that indicated FNA pretreatment improved the hydrolysis rate and biodegradation potential (Wang et al., 2013b). However, the VFA yield decreased nearly 23% with more FNA addition to 3.08 mg HNO_2-N/L, while the underlying mechanisms remain unclear. It is worth mentioning that a limited effect on hydrolysis and biodegradability of PS by FNA pretreatment was observed compared to that of WAS (Zahedi et al., 2016; Zhang et al., 2016), which was probably due to the different substrate components and the biocidal effect function of FNA.

2.3. Biological pretreatment methods

2.3.1. Enzyme pretreatment

Microorganisms hydrolyze biomass with the help of various extracellular enzymes. It is possible to improve the solubilization of particulate organic matter in sludge and further enhance VFA yield by addition of enzymes during the anaerobic fermentation process (Romano et al., 2009). Compared to physical and chemical pretreatment methods, enzymes hydrolyze sludge without production of refractory products.

For sludge biomass solubilization, amylase and protease or a combination of the two were widely tested, because carbohydrate and protein are major components of sludge. Among them, it seems that endogenous amylase showed higher efficiency in solubilization than endogenous protease or a combination of both (Yang et al., 2010; Yu et al., 2013). In addition to major biodegradable macromolecule substances, the existence of lignocellulosic materials in sludge that are complex and recalcitrant in structure also limit sludge biodegradation. Addition of cellulase and ß-glucosidase was found to increase the anaerobic biodegradability and methane production of sludge (Higgins and Swartzbaugh, 1986). Similarly, Roman et al. (2006) also indicated that the addition of a combination of cellulase and pronase resulted in 4-fold higher improvement in solid reduction compared to the control group during anaerobic digestion of primary sewage sludge. It is important that these enzymes are able to degrade organic substances mainly from EPS, while not providing a direct conversion from solid into the liquid phase (Ayol, 2005; Watson et al., 2004). Sesay et al. (2006) observed unremarkable cell lysis in sludge after addition of cellulase, α-amylase, and proteinase.

He et al. (2014), who investigated the effect of lysozyme for promoting WAS biodegradability, demonstrated that the ratio of SCOD to TCOD increased significantly with a lysozyme/TS ratio ranging from 5% to 15% (W/W, lysozyme weight/TS weight) and lysozyme incubation time from 0 to 240 min. Yu et al. (2013a) demonstrated that the addition of 10% (W/W) crude amylase extracted from strains was efficient in increasing the SCOD by 78.2% and the VFA concentration production after 7 hr by 129.6%. The increased VFA production of sludge with the help of enzymes mainly resulted from increased solubilization and their own digestion function (Christ et al., 2000; Yu and Fang, 2001).

Several factors, such as incubation time and environmental conditions, can influence enzyme activity (Sukumaran et al., 2009). More research is necessary to elucidate the strategies for enzyme dosing, including how and when the addition of enzymes to the anaerobic digestion system can improve the digestion rates and VFA yields of sludge. For instance, enzymes could be added as enzymatic solutions or bioaugmentation to treat sludge biomass before anaerobic fermentation, or added to the anaerobic digestion process (Romano et al., 2009). In short, biological pretreatment using enzymes is a mild and environmentally friendly approach to enhancing the hydrolysis rate and biodegradation of sludge, even though the high cost of commercial and/or low-purity enzymes is still a prime limitation for its wide application (Harris and McCabe, 2015).

2.3.2. Bio-surfactant pretreatment

Recently, bio-surfactants secreted extracellularly or as a fraction of the cell membrane by microorganisms (Zajic et al., 1983), such as glycolipids (e.g., rhamnolipid), lipopeptides (e.g., surfactin), saponin (e.g., tea saponin), and phospholipids, have been applied in sludge pretreatment over a wide range of temperatures, pH, and salinity (Ji et al., 2010; Liu et al., 2018a; Mayer et al., 1999; Xu et al., 2018; Zhang et al., 2010). As a novel pretreatment method, bio-surfactants can change the affinity between microbial cells and organic matter, thus enhancing the solubilization of EPS from the cell surface (Wang et al., 2011a). Moreover, some reports stated that the bio-surfactants can stimulate hydrolytic production of enzymes such as cellulase and xylanase during the fermentation process (Liu et al., 2006).

Research regarding the improvement of VFA production by bio-surfactants during anaerobic fermentation is still at a preliminary stage, but some exciting results have been published (Yi et al., 2013; Zhou et al., 2013b). Among them, rhamnolipids, a group of bio-surfactants, have been studied extensively for the promotion of organic solubilization and enhancement of VFA production from sludge. For example, Yi et al. (2013) indicated that addition of 0.3 g rhamnolipid/g dry sludge improved the hydrolysis of EPS approximately 4.8-fold after 6 hr, and the VFA yields almost tripled after 3 days compared to the blank test during the anaerobic fermentation of WAS without inoculum sludge. In addition, Zhou et al. (2013b) reported that the optimal dosage of rhamnolipid at 0.04 g/g TSS resulted in 3.24-fold higher VFA production than that in the blank after 96 hr. Besides, the mechanisms of the main three types of bio-surfactants (surfactin, rhamnolipid and saponin) used for VFA production of sludge were investigated (Huang et al., 2015). Surfactin mainly increases the dissolution of organic matter to reach a high VFA accumulation level. Rhamnolipid is a suitable candidate to increase VFA production and also inhibits methanogenesis. However, it simultaneously slows down the metabolism of other microorganisms in sludge, which could undesirably decrease dehydrogenase and acetate kinase activities and further decrease acetate production (Huang et al., 2015). In addition, a small part of rhamnolipid and saponin that both have glycosyl groups could be hydrolyzed into saccharides, which were used as substances for VFA accumulation. To achieve the same VFA yield during anaerobic fermentation, the required dosage of surfactin was 2.5- and 5.0-fold less compared to rhamnolipid and saponin, respectively (Huang et al., 2015; Zhou et al., 2013b).

Other bio-surfactants may be more effective; however, little information about the role of different types of bio-surfactants in the promotion of VFA production has been introduced (Huang et al., 2015; Zhao et al., 2015b; Xu et al., 2019). Therefore, it is necessary to screen out suitable bio-surfactants for sludge from different sources and further investigate mechanisms of the enhancement by different bio-surfactants. Some works have been carried out at lab scale, with few works at pilot and full-scale due to the high cost of commercial bio-surfactants. Nonetheless, bio-surfactant production from inexpensive substrates and effective microorganisms, such as agro-industrial wastes, could be considered as a promising strategy, and could mitigate agricultural and industrial waste treatment issues.

2.4. *Combined pretreatment*

The performance of sludge fermentation depends on several factors such as sludge type, process parameters and so on. Due to the intrinsic disadvantages and limitations of individual pretreatment methods, therefore, researchers have investigated combined pretreatments coupling two or more pretreatment methods to create synergistic effects on the hydrolysis and even acidification of sludge (Feng et al., 2014; Liu et al., 2018b; Tan et al., 2012).

For example, a combination of alkaline and microwave pretreatments was carried out to produce VFA by anaerobic fermentation from WAS, resulting in about 80% enhancement of VFA yield and reduction in fermentation time (Yang et al., 2013). In this combined pretreatment, the alkaline solution was initially added into sludge to break down the floc structure and weaken the cell walls of the bacteria, rendering them more susceptible to lysis by sequent microwave pretreatment. These processes can then generate a synergetic effect for hydrolysis and acidification, thus resulting in shortening the time needed for high VFA accumulation. Moreover, comparing the effects of thermo-acid, thermo-alkaline, ultrasonic-alkaline and ultrasonic-acid combined pretreatment techniques on the solubilization and subsequent acidification efficiency of WAS, the thermo-alkaline and ultrasonic-alkaline pretreatment methods showed greater efficiency in VFA production due to significant improvement in WAS solubilization by 60.2%–61.6% (Liu et al., 2009). As discussed in Section 2.1, thermal effects during ultrasonic pretreatment process lead to improvement in hydrolysis and biodegradability.

Another pretreatment combination method employing a surfactant and biological enzyme was examined in which the added surfactant caused a marked increase in the aqueous solubility of additional hydrolysis enzymes, leading to enhanced hydrolysis and acidification (Luo et al., 2011). The combination was more effective in the promotion of sludge hydrolysis and showed better VFA production performance than sole SDS or sole enzyme addition.

As mentioned above, combined pretreatments usually are beneficial because of synergetic effects on improvement of the hydrolysis rate, which is an indicator for higher VFA production. However, the detailed mechanisms of synergetic effects are still missing and unclear. A techno-economic feasibility study would be necessary to identify the optimal pretreatment method (Cao and Pawłowski, 2012; Ruffino et al., 2015), because occasionally the combination of pretreatment practices increases the consumption of energy and chemical reagents.

3. Co-fermentation

Co-fermentation, the simultaneous fermentation of two or more substrates, is a feasible alternative to enhance anaerobic fermentation for VFA production. The main advantages of this technology are as follows: (1) increase in organic content; (2) dilution of inhibitory and/or toxic compounds; (3) balancing the C/N ratio; (4) reduction of reactor volume; (5) improvement of buffer capacity; and (6) optimization of rheological qualities (Banerjee et al., 1998; Gómez et al., 2006; Li et al., 2018; Mata-Alvarez et al., 2000). Under these circumstances, synergistic effects may be achieved; this means that co-fermentation produces more VFA than the addition of the VFA produced in both digestions separately. Sewage sludge is a feedstock characterized by a relatively low C/N ratio (by weight), ranging from 6 to 9, and high buffer capacity (Astals et al., 2013; Silvestre et al., 2011). In addition to low carbon biodegradability, sludge can also evolve ammonia, which is toxic to microorganisms and can even completely halt the fermentation (Rughoonundun et al., 2012). Therefore, to enhance fermentation efficiency, it would be advantageous to combine co-substrates with easily biodegradable organic matter and low alkalinity, such as agricultural residues and municipal solid wastes (Li et al., 2013; Rughoonundun et al., 2012). Table 7 is a brief summary of co-fermentation of sewage sludge with other organic substrates to improve the performance of the anaerobic process for VFA production (Banerjee et al., 1999; Del Rio et al., 2014; Chen and Wu, 2010; Maharaj and Elefsiniotis, 2001).

Unlike the typical pretreatment methods, co-fermentation speeds up the hydrolysis rate and biodegradation mainly by adjusting and optimizing the microbial metabolism without direct floc breakdown or cell lysis (Huang et al., 2016; Krupp et al., 2005; Wu et al., 2016). Examining the effect of different C/N ratios on VFA production from WAS combined with carbon-riched agricultural residues, Guo et al. (2015) reported that the maximum VFA yield of 486.6 mg COD/g VSS was obtained for straw-conditioning with a high C/N ratio (=20), which was a 3-fold increase over a blank test due to the remarkable accelatation of hydrolysis and conversion. Similarly, co-fermentation of sludge with food waste also led to more than a 5-fold increase in VFA yield compared to sludge alone, at the optimal C/N ratio of 22 under alkaline conditions (Chen et al., 2013b). Moreover, Huang et al. (2016) reported that a novel agricultural residue, named henna plant, not only adjusted the C/N ratio but also released an efficient electron-shuttling mediator (lawsone) which has positive electron-shuttling and -transformation effects that improve VFA production.

Although there is no clear link between different types of substrate and VFA composition, variations in substrate composition in co-fermentation influence the VFA spectrum, acetate being the predominant product in most cases (Ma

Table 7 – Co-digestion of sewage sludge with other organic substrates for volatile fatty acid (VFA) production.

Feedstock	Result	VFA yield	Influencing factor	Reference
Dewatered excess sludge + food waste	Maximal VFA production of 29 g/L	392 mg/g VSS	Nutrients balance	Chen and Wu (2010)
Waste activated sludge + agricultural residues	Increased 3-fold VFA yield	487 mg COD/g VSS	C/N ratio	Guo et al. (2015)
Waste activated sludge + corn straw	Increased by 96% VFA yields	246 mg VFA (as COD)/g VS	Nutrients balance	Zhou et al. (2013a)
Waste activated sludge + kitchen waste	Increased by about 5 times VFA production	670 mg COD/g VS	C/N ratio	Chen et al. (2013b)
Waste activated sludge + pretreated bagasse	Increased by about 3 times VFA production	360 mg/g VSS	C/N ratio	Rughoonundun et al. (2012)
Waste activated sludge + food waste	Increased by about 4 times VFA production	424 mg/g VSS	Nutrients balance	Li et al. (2013)
Aerobic granular sludge + primary sludge	Increased by about 40% VFA production	118.4±5.8 mg COD/g VSS	Synergistic hydrolysis	Del Rio et al. (2014)
Primary sludge + starch-rich waste water	Increased by 33% VFA production	124 mg COD/g VSS	Increasing the load of biodegradable organic matter	Maharaj and Elefsiniotis (2001)
Primary sludge + potato-processing wastewater	Increased by 39% VFA production	394±93 mg/g VSS	Increasing the load of biodegradable organic matter	Banerjee et al. (1999)

et al., 2017; Rughoonundun et al., 2012; Zhou et al., 2013a). Another important fact is that protein-rich waste streams such as primary sludge benefit the enhancement of odd-numbered VFA production (Ma et al., 2017). Above 78% of propionate was yielded from primary and activated sludge (Min et al., 2005; Zhu et al., 2008). However, due to different substrate components and operating conditions such as pH and inoculum ratio, it is hard to obtain consistent results. For instance, Zhou et al. (2013a) investigated the effect of feedstock proportion on VFA production from anaerobic co-digestion of WAS with corn straw, and the results showed that the increase in the fraction of corn straw in the feedstock led to higher production of propionate. Besides, in the co-fermentation of sewage sludge with pretreated bagasse (Rughoonundun et al., 2012), the results showed that the percentage of acetate rose along with increasing amounts of pretreated bagasse in the mixture of substrates.

In short, this demonstrates that the anaerobic co-fermentation of sewage sludge with one or more substrates is a promising approach for the enhancement of VFA yield and the regulation of VFA spectrum. However, it is worth noting that the operational conditions, such as pH and alkalinity, as well as trace elements, are also important for the optimization of the C/N ratio to determine the best combination (Wang et al., 2011b). More specific data are required to identify the optimal operational conditions and elucidate the ways in which mixed substrates impact on the VFA composition. Mixtures of sludge and agricultural residues and municipal solid wastes have been the most reported mixed substrates (Yuan et al., 2012), while guaranteeing a stable supply and collection, as well as the transportation cost of co-substrates to anaerobic digestion plants, should be considered.

4. Summary and prospects

Anaerobic digestion of sewage sludge for methane production can be regarded as a traditional and classic method that combines solid stabilization and resource recovery (Appels et al., 2008). In recent years, an alternative approach has aimed at converting sewage sludge into VFA instead of methane production, which has gained growing attention. The driving force behind this trend is that VFAs are considered as high value-added products due to their high commercial value and wide applications (Agler et al., 2011; Alloul et al., 2018; Lee et al., 2014).

As a series of biochemical reactions, anaerobic fermentation can be affected by many environmental factors and operating parameters such as pH, temperature, retention time and so on. Many studies on VFA production from sludge under various conditions have been carried out (Chen et al., 2013a; Li et al., 2011, 2013; Tong and Chen, 2007; Wang et al., 2013a; Zhang et al., 2009) on the VFA yield, VFA spectrum, and micro-community distribution in the fermentation processes. The operational pH is of special interest from an application point of view, since the addition of basic chemicals for pH control can change the type of fermentation, further boosting the VFA yield and thus improving the economic feasibility of large scale implementation. An overall review on the microbial community of fermenters fed with sludge (or with co-substrate) would be conducive to our further understanding of the underlying mechanism of VFA production and obtaining the desired VFA spectrum by manipulating operational conditions. Furthermore, many studies have been conducted to understand the effects of trace elements on biogas production from sludge, while they have mainly focused on their effects on methanogenesis rather than hydrolysis and acidification (Hendriks et al., 2017). Hence it is of great interest to examine the utilization of trace elements to enhance the hydrolysis and acidification of fermentation at the enzymatic level, leading to VFA accumulation and then simultaneously inhibiting methanogenesis, due to the substantial differences in the metabolisms of acidifiers and methanogens.

Hydrolysis is regarded as the rate-limiting step in anaerobic digestion, hence the enhancement of hydrolysis by using different pretreatment methods is a positive step for improvement of VFA production. However, direct comparisons of the VFA production by various pretreatment methods from different studies are unfair, because they depend upon different sources and types of sludge, as well as the process parameters in the fermentation process. It should be noted that all pretreatment practices have advantages and disadvantages. A general comparison of the advantages and disadvantages of various pretreatment methods on sewage sludge is summarized in Table 8.

In general, both physical and chemical pretreatments have shown high efficiency in sludge disintegration and VFA production, with less process time consumption. However, most of these methods produce by-products that may inhibit the anaerobic fermentation process. It has been found the residue compounds inhibited ethanol production (Parawira and Tekere, 2011). To the best of our knowledge, however, there are no similar reports on the effect of the physical and chemical pretreatment by-products on VFA production. Therefore, it is necessary to further clarify the impacts of by-products from pretreatment methods on the consortium of microorganisms involved in VFA production and consequent processes. Unlike physical and chemical pretreatment methods, biological pretreatment usually consumes much lower energy, and it works under much milder environmental conditions, so that few inhibitors are generated. However, it is not as efficient as physical and chemical pretreatment methods and, moreover, requires longer pretreatment time and higher commercial production cost, which hampers its application. Considerable numbers of studies have also investigated the synernegistic effects of the combination of several different pretreatment methods and substrates. In fact, selection of a proper method to increase VFA production from sludge should at least consider local regulations and economic conditions. High-thermal pretreatment seems one of the ideal options if sterilization or a Class A standard is required.

Table 8 – Main advantages and disadvantages of typical pretreatment methods for VFA production.

Pretreatment method	Advantages	Disadvantages
Ultrasonic	Particle size reduction; Scalability; No risk of recalcitrant compounds formation	High energy cost
Thermal	High solubilization improvement	Possible formation of complex substrates that are difficult to biodegrade
Alkaline/acids	Low capital costs; Short reaction time	Corrosion of equipment; Chemical contamination
Ozonation	High solubilization improvement; Short reaction time	High energy cost; Possible formation of less biodegradable byproducts
Enzymes	Low energy demand; Scalability	High cost
Thermo-chemical	Lower energy demand than thermal alone	Risk of inhibitors formation
Mechanical–chemical	High solubilization improvement; Short reaction time	High capital cost than alone

However, many challenges and bottlenecks facing VFA production from sewage sludge still hinder its application. Firstly, merely using COD solubilization is improper to evaluate the efficiency of VFA production from sludge by different pretreatment methods or co-fermentation. The sludge biodegradation assays using standard biomethane potential (BMP) tests can evaluate the maximum methane production (Holliger et al., 2016). Analogous to the BMP tests, it would be interesting to establish a standard method to evaluate the maximum VFA production from various substrates.

Besides, cost-efficient pretreatment methods need to be considered to further solubilize the sludge and increase fermentative VFA yields. Alkaline and mild-thermal pretreatments are likely to be promising methods due to their features of short reaction time and low investment cost. Moreover, it has been reported that FNA, a byproduct of wastewater treatment created through nitritation of the anaerobic digestion liquor, has been shown to cause significant elevation of VFA production, which is a promising method (Wang et al., 2013b). From this aspect, assessment of the cost-effectiveness of each technique should be provided in the future. Besides, the imbalanced nutrition in sludge is another important factor which limits the anaerobic biodegradability potential for VFA production. To overcome these issues, co-fermentation of sludge with C-rich substrates would be a feasible solution.

Additionally, control of the VFA spectrum is critical because it influences the consequent application processes, such as PHA production and chain-elongation process (Lee et al., 2014). However, most published studies have focused on improving VFA yields while fewer have paid attention to investigating how to control the product spectrum in reactors under different operation conditions. In the future, research regarding oriented VFA production, such as odd-number VFA production, would probably be a promising field.

Moreover, as an important precursor, it is necessary to deeply consider VFA production together with consequent utilization, not simply VFA production itself. Currently, recovering VFA from sewage sludge is still a challenge in WWTPs. Apart from feeding BNR processes, more novel and potential applications of the high-VFA stream produced from the sludge should be carried out, for instance, using the stream to feed microbial fuel cells to generate electricity or to feed bio-electrochemical cells to produce biofuels (Kondaveeti and Min, 2015), which can be used to meet the energy demand of the facilities of WWTPs. Moreover, converting soluble acids produced into higher valued products such as PHA or media fatty acids production would be another promising method (Spirito et al., 2014).

5. Conclusions

The slow hydrolysis step still limits fermentative VFA production from sewage sludge. Different pretreatment methods degrade EPS and even trigger cell lysis, resulting in particle reduction and release of organic matter, improving the hydrolysis rate and biodegradation of sludge. In some cases, combined pretreatment can create a synergistic effect on the hydrolysis and acidification of sludge, although this can be more energetically expensive. Co-fermentation of sewage sludge with substrates with a higher C/N ratio can effectively optimize the initial C/N ratio and adjust the metabolism of the microbial community during anaerobic fermentation, mainly speeding up the hydrolysis rate and biodegradation of the organic matter, not resulting in direct floc breakdown or cell lysis. The yield and composition of the VFAs generated during the anaerobic fermentation of sewage sludge are influenced by several factors, such as the type of sludge and operating parameters (pH, temperature and others). However, the existing data regarding the effects of operating parameters on VFA composition are often contradictory, since different operation conditions are employed in each research study. Hence, it is necessary to develop a standardized protocol for the assessment of VFA production in the future to help to compare different pretreatment methods and their effects on VFA yield and composition. Moreover, to better understand the underlying mechanisms of each approach, more attention should be directed to the conversion of specific components, not merely into SCOD. More importantly, an assessment of the cost-effectiveness of each technique should be provided from technical, economic and environmental perspectives in the future. Furthermore, more attention still needs to be paid to the conversion of, and structural changes in, the different complex components of waste activated sludge when various pretreatment methods are applied.

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