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Biomethane potential test for rapid assessment of anaerobic digestion of sewage sludge: co-digestion with glycerol and trace organic removal

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This thesis is presented as part of the requirements for the award of the Degree of Master of Philosophy from the University of Wollongong

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CERTIFICATION

I, Thanh Nguyen, hereby declare that this thesis, submitted in partial fulfilment of the requirements for the award of Master of Engineering by Research Degree, to the school of Civil, Mining and Environmental Engineering, Faculty of Engineering, University of Wollongong is wholly my own work unless otherwise referred or acknowledged. The document has not been submitted for qualification at any other academic institution.

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ABSTRACT

Anaerobic digestion (AD) is the most widely used treatment process for sewage sludge stabilisation over concerns of public health. In addition, the production of methane (CH_4), a renewable fuel, has also shaped the prospective of AD within the context of energy security and global warming. This dissertation thus aimed to evaluate two main aspects of the AD process of sewage sludge. First, the potential of enhancing biogas production was assessed when co-digesting sewage sludge with glycerol. Glycerol is a by-product of biodiesel production and then rich in organic carbon. Second, the capacity of removing trace organic compounds (TrOCs) was examined.

Results reported here suggest the stability of anaerobic conversion determined the ultimate CH_4 yield, greatly affecting the assessment of CH_4 potential. Alkalinity buffer and inoculum over substrate (I/S) ratio were demonstrated to be important factors in maintaining steady state of the AD process. The addition of $NaHCO_3$ resulted in an increase of CH_4 production of sewage sludge that could be ascribed to well-buffered conditions for methanogens. A significant enhancement in CH_4 yield from sewage sludge was achieved with an increase of I/S ratio from 1/9 to 1/1. Adequate quantity of inoculum for degrading substrate was responsible for such improvement. Moreover, no disruption of CH_4 production was found since only 0.25% and 0.5% glycerol of digested sludge (by volume) was applied. The obtained ultimate CH_4 yields of three types of glycerol were comparable, indicating that any glycerol was possibly used as a potential co-substrate of sewage sludge. Additionally, the identification of appropriate values of alkalinity and I/S ratio for an optimal CH_4 production was dependent on key characteristics (i.e. pH and alkalinity) of sewage sludge and glycerol.

Experimental results from anaerobic co-digestion of raw primary sludge and glycerol show that the addition of 0.5% and 1.0% of glycerol to raw primary sludge (by volume) could improve the anaerobic sludge conversion in terms of the daily and cumulative CH_4 production. Carbon-rich content and high solubility of glycerol led to an increase only within the first seven days. Despite the buffer supplementation, the instability of the AD process was clearly observed in this batch test through characteristics of the feeds and digestates (acidic pH and low alkalinity for example),

suggesting a shock of organic loading due to insufficient inoculum and extra organic matter from glycerol. The feasibility of glycerol as a co-substrate of raw primary sludge was satisfactory to give rise to CH_4 generation. On the other hand, excessive addition of glycerol as a co-substrate may destabilise the AD process, and thus be counter-productive.

The removal of TrOCs by AD was evaluated. The evolution of pH and alkalinity as well as CH_4 yields obtained from experiment data and kinetic analysis indicated a steady state of methanogenesis in TrOC-spiked bottles after 1.6 days. In a two-phase matrix (i.e. sludge and water), TrOCs could partition (adsorb) to the solid phase as well as the water phase. Their distribution in these phases was examined using a two-phase fate model, which considers mass transfer (i.e. sorption and desorption). In this study, the removal of the investigated TrOCs is defined as anaerobic biodegradation. Their various removal efficiencies were consistently related to their molecular properties in terms of functional groups rather than other physicochemical properties. Compounds with the inclusion of halogen, methoxy and amine/amide groups exhibited an effective removal while low or no removal efficiencies were observed in compounds possessing alkyl functional groups. Regarding compounds possessing high biodegradability (k_b> 0.001 L/gVS.d), water-solid partition coefficient (k_p) determined from the proposed two-phase fate model could describe their distribution in the sludge phase better than their hydrophobicity (in terms of log *D*). For biologically persistent compounds (k_b< 0.001L/gVS.d), sorption properties, which can be predicted by log *D* values, were responsible for the fate of organic contaminants in sludge matrix.

An economic design at laboratory scale of biomethane potential testing could rapidly assess the AD of sewage sludge. Results also demonstrated increased methane yields when using glycerol as a co-substrate of sewage sludge and trace organic removal efficiency under anaerobic conditions. These findings are of great importance for scaling up AD facilities in real wastewater treatment plants.

Keywords: anaerobic digestion, anaerobic co-digestion, biomethane potential, sewage sludge, glycerol, trace organic compounds, sorption.

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LIST OF ABBREVIATION

AD	Anaerobic digestion
BMP	Biomethane potential
COD	Chemical oxygen demand
COD_s	Chemical oxygen demand – soluble fraction
COD_t	Chemical oxygen demand – total sample
EDCs	Endocrine disrupting chemicals
GC–MS	Gas chromatography – mass spectrometer
I/S	Inoculum over substrate
MBR	Membrane bioreactor
PPCPs	Pharmaceutical and personal care products
SMA	Specific methanogenic activity
SPE	Solid phase extraction
TrOCs	Trace organic compounds
TS	Total solids
VFAs	Volatile fatty acids
VS	Volatile solids
WWTPs	Wastewater treatment plants

1 INTRODUCTION

1.1 Background of the study

Since the 1990 North Sea Conference, where international agreement took place to phase out the discharge of raw sewage sludge at sea, the treatment of sewage sludge from wastewater treatment plants prior to environmental disposal has become a norm in developed countries. There are several techniques for the treatment and management of sewage sludge, including landfilling, incineration, composting, and anaerobic digestion (AD) process [1, 2]. Among them, AD is the most commonly used technique since biogas, which is a valuable form of renewable energy, can be extracted from sewage sludge.

In recent years, the application of AD for sewage sludge treatment has grown rapidly around the world. AD involves several sequential biochemical processes. Each stage is consistently performed by a specific bacterial group in the absence of oxygen [3]. The production of biogas in AD offers several significant advantages over other alternative technologies. These include biogas production, nutrient recovery, and reduction of waste organic content and pathogen agents [4-6]. Recent development of high-rate anaerobic systems has made AD an attractive technology for managing organic wastes from agricultural production, as well as municipal and industrial wastewater treatment [7]. AD can be applied to a range of feedstocks including solid wastes from husbandry, food processing, and wastewater sludge [8, 9].

Sewage sludge can be described as a byproduct mixture of solids and water from wastewater treatment [10]. By applying different treatment processes, the resulting sewage sludge types completely differ in their characteristics. Constituents of sewage sludge in terms of carbohydrate, lipids and protein are highly variable depending on their origin [2]. The presence of significant concentrations of nitrogen, phosphorus and potassium in sewage sludge make it possible for fertilising soil since these elements are essential for plant growth.

AD instability is caused by fluctuations in organic loading rate, heterogeneity of wastes or excessive inhibitors. Towards improving AD performance in biogas production and accelerating the microbial activity for higher quality of biosolids, various environmental conditions should be meticulously controlled. Additionally, several studies have demonstrated that the hydrolysis phase is a rate-limiting step, and directly affect the performance of AD [11-14].

Co-digestion of sewage sludge with higher degradable carbon source has become an effective solution to enhance anaerobic degradation and biogas production. Anaerobic co-digestion involves the simultaneous digestion of a homogenous mixture of two or more substrates and has been promoted very recently in many WWTPs [15]. Not only does this process accelerate the hydrolysis and biogas yield, it also offers many other benefits, including dilution of potential toxic compounds, the supply of missing nutrients, synergistic effects of microorganisms, increased load of biodegradable organic matter, economic advantage of sharing equipment, and better biogas yield [16, 17]. Of the organic waste materials investigated in the literature, crude glycerol has been reported to be an ideal co-substrate for the anaerobic mineralisation of sewage sludge [18-20]. Crude glycerol is highly soluble and rich in easily degradable components.

Given that both biogas production and digestate quality from the anaerobic treatment of sewage sludge were of great importance, considerations must also be given to the fate of trace organic compounds (TrOCs) available in sewage sludge. The accumulation of certain emerging organic compounds at trace levels onto sewage sludge has been widely observed from WWTPs since adsorption is one of main mechanisms responsible for their elimination during several wastewater treatment processes [21-23]. For example, Samaras et al. [21] determined that while biodegradation/biotransformation was responsible for trace organic removal from wastewater the sorption tendency onto sludge particles contributed to the removal of nonylphenol and triclosan from wastewater.

Studies reporting on the fate of these compounds during AD of sewage sludge have aroused question over their occurrence in sewage sludge and their subsequent impact on agricultural applications of biosolids. Unlike wastewater, sewage sludge was regarded as a two-compartment matrix, including water and solid phases. As such, under anaerobic sludge treatment process, every compound could undergo two main routes, eliminated by abiotic and/or biotic removal mechanisms and/or accumulated onto sludge particles. Several studies have indicated significant removal efficiencies of some TrOCs including surfactants, pharmaceuticals, and personal care products by AD treatment [22-27]. The author mainly attributed such efficient removal performance to anaerobic biodegradation. Nonetheless, the behaviour of TrOCs in two phases of sewage sludge and the relative impact of their behaviour on

biodegradability remained unclear. An extension of investigations on compounds belonging to different usage groups with diverse physicochemical properties was of necessary consequently. On the other hand, co-digestion of sewage sludge with readily biodegradable substrates could enhance their anaerobic biodegradation although to date there have been very little evidence to substantiate this hypothesis.

1.2 Scope of study

The increasing demand of renewable source of energy and adequate quality of biosolids has provoked a great deal of work to formulate the feasible treatment processes applied in WWTPs. In addition to sewage sludge stabilization, AD has been known to produce biogas, which is a renewable fuel. Using organic materials as co-substrate of sewage sludge is expected to enhance the efficiency of anaerobic digesters. Furthermore, a more comprehensive understanding of key physiochemical properties of the substrate, operational conditions, and biogas potential is of great necessary prior to any large-scale operations.

The accumulation of TrOCs in biosolids is also a great challenge because they severely affect the suitability of biosolids for land applications. Although the removal of TrOCs by aerobic treatment has been extensively studied, little attention has been paid to the anaerobic treatment. The assessment of their fate throughout the anaerobic fermentation by batch-mode biomethane potential (BMP) tests can provide valuable information on elimination capacities at different redox conditions.

1.3 Research objectives

BMP batch tests are conducted to evaluate the potential of the AD process in producing biogas and treating TrOCs in sewage sludge. The outcomes will then facilitate the performance of anaerobic digesters in water utilities in Australia and around the world. The specific objectives of this research are:

- Characterise sewage sludge and glycerol for parameters relating to the AD process;
- Assess the potential of methane (CH_4) production of each sewage sludge and glycerol;
- Evaluate the generation of biogas during anaerobic co-digestion of sewage sludge with glycerol; and

- Evaluate the removal of TrOCs accumulated in sewage sludge from wastewater treatment processes by the AD process.

1.4 Expected outcomes

The main expected outcome of this study is the better performance in terms of CH_4 yield when co-digesting sewage sludge with other organic waste materials in lab-scale batch experiments. Prior to this, data regarding the varied compositions of sewage sludge and other organic waste materials will be identified; and the BMP of each substrate is assessed. Another important outcome will be the extent of elimination of some TrOCs by the anaerobic conversion.

1.5 Thesis outline

This thesis consists of seven chapters systematically shown in Figure 1. Chapter 1 gives an introduction of background and research objectives. Chapter 2 is a thorough and updated review on the literature of AD and its applications on the co-digestion together with publications of the fate of certain persistent compounds. Chapter 3 describes the research methods to fulfil specific objectives clarified in Chapter 1. From Chapter 4 to Chapter 6, experimental results and data analysis are presented. Chapter 7 will draw conclusions of this whole study and recommendations for future studies.

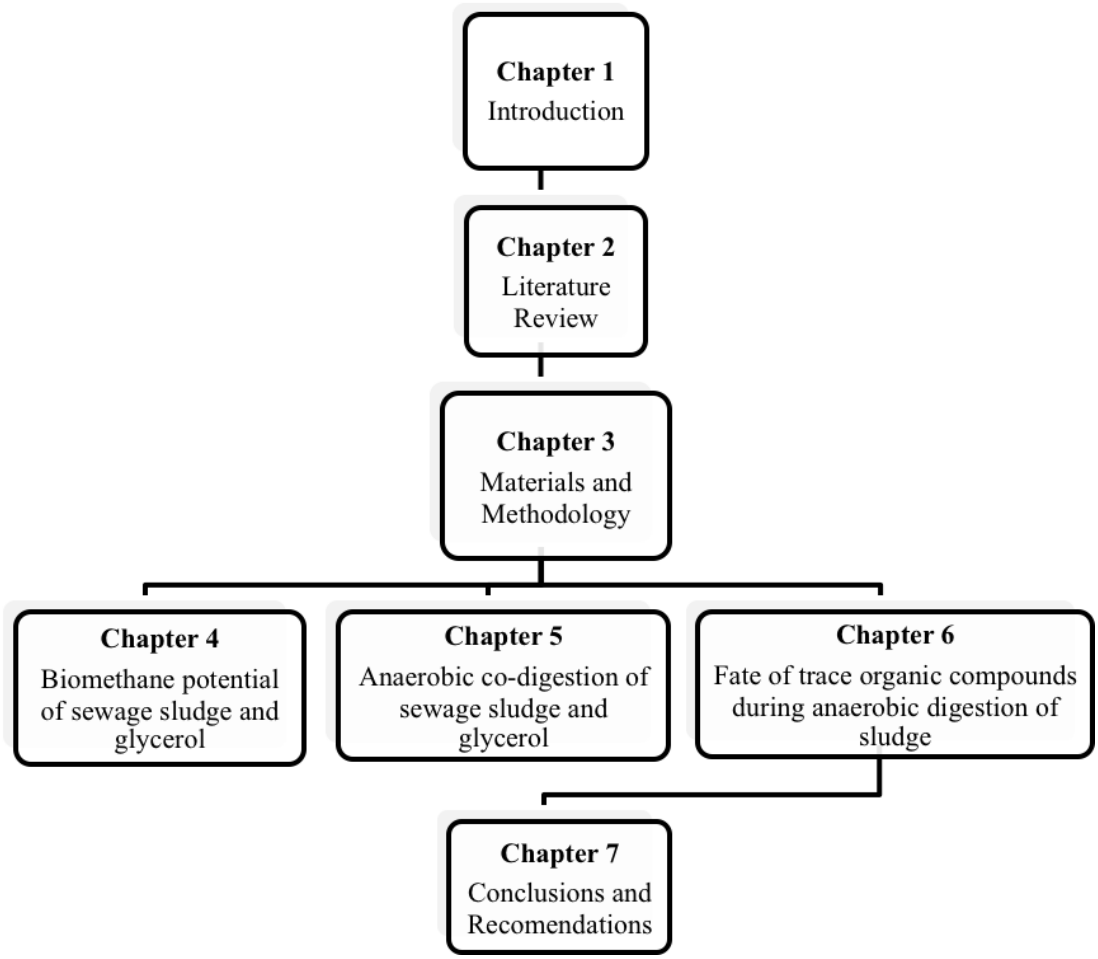


Figure 1. The descriptive structure of thesis.

2 LITERATURE REVIEW

This chapter provides an overview of the current knowledge regarding anaerobic co-digestion of sewage sludge and other organic waste materials. The AD process of sewage sludge is firstly presented and discussed. This is followed by a comprehensive review of CH_4 production by anaerobically co-digesting sewage sludge with other substrates. In addition, the behaviour of TrOCs during AD is also summarised.

2.1 Sewage sludge

In the effort to improve effluent quality, WWTPs are built and upgraded. While these plants can produce high effluent quality, sludge disposal remains an underlying issue. These include the high cost of sludge treatment, which makes up more than 50% of total wastewater treatment cost [28], and potential risks associated with sludge disposal for the environment and human health [29].

Sewage sludge is a mixture of solids and semi-solids removed from the liquid stream of WWTPs [30]. A more restricted definition is "a residual solid from sewage plants treating domestic and urban wastewaters and from other sewage plants treating wastewater of a composition similar to domestic and urban wastewaters" [10].

2.1.1 Types of sewage sludge

To assess options for sludge treatment and disposal, it is necessary to investigate different kinds of sludges and their origins. A typical sewage treatment plant includes preliminary, primary, secondary and tertiary processes [2]. During preliminary treatment, large debris (sticks and papers for example), are removed and these do not add to sewage sludge. On the other hand, residues from all the other processes are collected as sludge (Figure 2).

Primary sludge is from the primary treatment process (commonly known as sedimentation), containing high total solids (TS) content. The characteristics of primary sludge varies considerably depending on the initial compositions of wastewater, the efficiency of primary sedimentation and the usage of chemicals in sedimentation, like coagulant aids [31]. Primary sludge can consist of oil, grease, vegetable materials, faecal materials, papers, sanitary and medical waste, kitchen wastes, and a variety of pathogens [32].

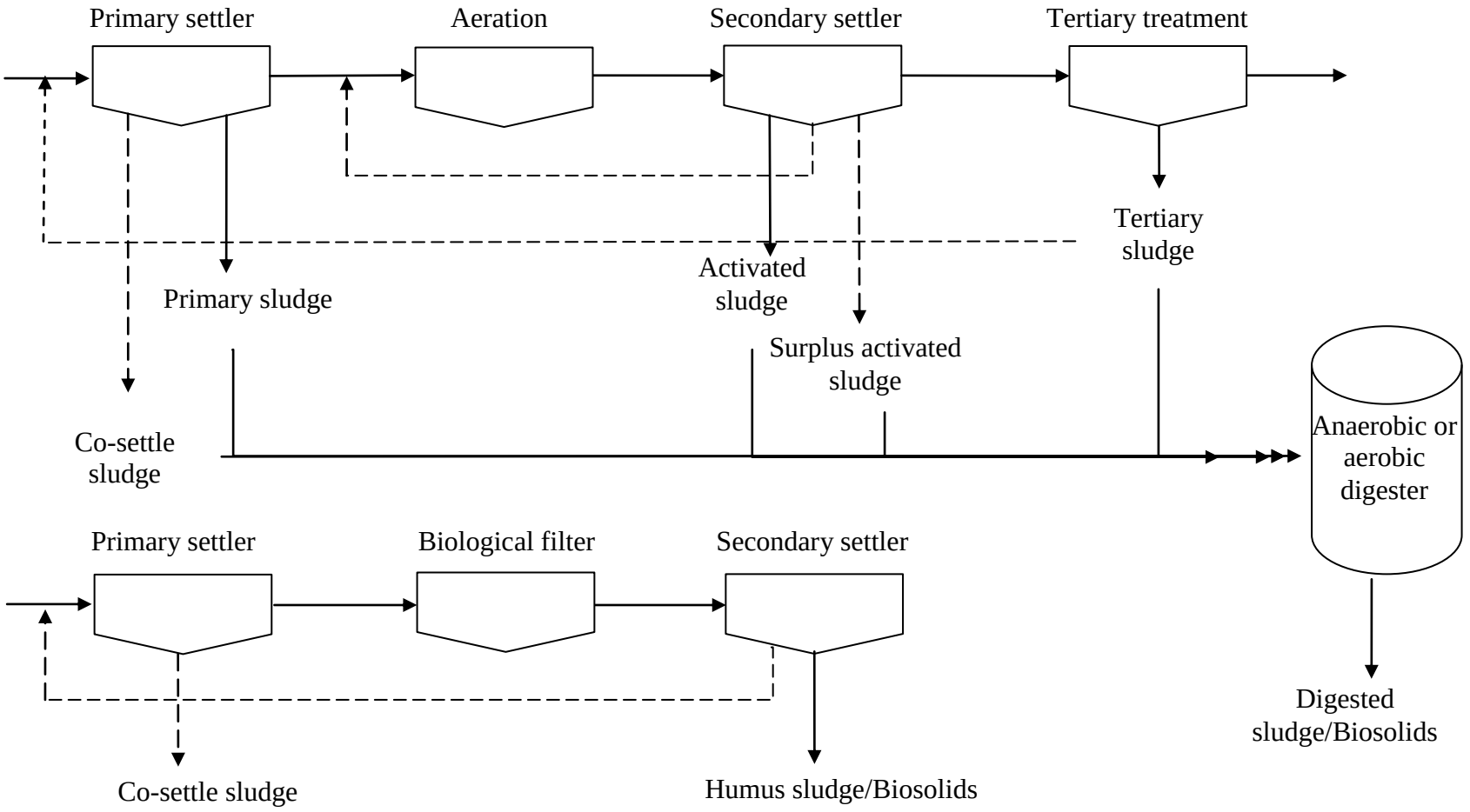


Figure 2. Different types of sewage sludge (modified from [10, 33]).

Treatment processes such as activated sludge process, trickling filter and rotating biological contactors result in humus sludge or biological sludge [30]. Humus sludge is the settled product from soluble waste in the primary effluent. This is a mixture of microorganisms: sloughed bacteria and fungus under living or dead remains. Humus sludge has an earthy smell and dark brown in colour.

Humus sludge from biological aerated filters and their variations, which have different types of biological media, share certain characteristics with activated sludge. In practice, humus sludge is returned to co-settle with primary sludge in the primary settler.

Activated sludge is removed from the activated sludge process. Main components of activated sludge are flocculated and synthesised solids and microorganisms [10]. Due to the rate of recycling and other factors, activated sludge has low TS (1%) with the colour ranging from grey, dark brown to black.

In the tertiary treatment step, the resulting sludge is called tertiary sludge. It has fractions in common with the secondary sludge, which remains in the effluent of the secondary treatment step and removed in the tertiary step. This sludge is normally transferred to primary tanks and co-settle with primary sludge due to its small amount.

Digested sludge, known as biosolids, is the product of biological digestion. This process can be performed in a reactor with or without the presence of oxygen, corresponding to the anaerobic or aerobic digestion processes. Biosolids contain nutrient [34] thus should be considered as a resource. They may also contain pathogens, which must be carefully managed for public health protection. Biosolids classification is based on contaminant and stabilization grades. Once these grades are evaluated, the beneficial use of biosolids can be divided into three categories: Unrestricted, Restricted and Not Suitable for Use [35].

With respect to the pathogens, biosolids are classified as either Class A or Class B according to the final part 503 regulations [34]. Class A biosolids contain a small amount of pathogens. As such, sewage sludge must undergo treatment methods, including composting, drying, and heat treatment, thermophilic AD process to achieve Class A standards. Class B biosolids have a small level of pathogens and less stringent requirements of treatment. Technologies, such as heating, composting,

digestion and pH adjustment, are in use to reduce the pathogen level to the point that protects public health and environment.

There are other kinds of sludges resulting from other treatment processes. One of them is physicochemical sludge coming from the physicochemical treatment of wastewater. It is composed of flocs produced by chemical treatments like coagulants and flocculants [33].

Combination of different sludge types is commonly utilised in sludge treatment. This could be elucidated with diverse characteristics and compositions of mixed sludge, facilitating downstream treatment processes. Regarding AD, the composition of sewage sludge is a mixture of primary and secondary sludge [36-38].

2.1.2 Constituents of sewage sludge

It is necessary to know characteristics of sewage sludge for its effective treatment and disposal. In general, sludge may include volatile, organic solids, nutrients, pathogens, metals, organic pollutants and water [28, 39]. Table 1 summarises some qualitative analyses of sludge constituents from the literature.

TS content affects the ability of sludge transference in the sewerage system. The higher amount of TS, the more difficulty sludge flow gets. Therefore, it is needed to remain sludge in liquid state, which will make sludge flow easily from a vessel and through pipes. Sewage sludge should be characterised for TS content prior to any sludge treatment processes.

The value of TS content after treatment can change depending on different treatment methods. After thickening, TS content of sludge will increase up to 9%; and reach 25 – 35% after mechanical dewatering [33].

The solid content of sludge has 59 – 88% of volatile solids (VS) on dry weight basis. VS content mainly contains organic compounds of animal or plant origin. It is defined as the mass of solid materials that can be lost through evaporation or oxidation at 550 °C.VS is an important parameter of the odour problem of sludge; thereupon, the reduction of VS is one of the main objectives in sludge treatment. A series of treatment methods, including AD, aerobic digestion, composting and incineration, are used to minimise the VS content [1]. AD can biologically convert around50% of VS to biogas.

Table 1. Typical constituents of different types of sludge [2, 10].

Constituent	Unit	Type of sludge			
		Untreated primary sludge	**Digested primary sludge**	**Activated sludge**	**Co-settled sludge**
Total solids	%	2.0 – 8.0	6.0 – 12.0	0.83 – 1.16	nd
Volatile solids	% of TS	60 – 80	30 – 60	59 – 88	nd
Grease and fat					
Ether soluble	% of TS	6 – 30	5 – 20	nd	
Ether extract		7 – 35	nd	5 – 12	14.7
Protein	% of TS	20 – 30	15 – 20	31 – 41	32
Total nitrogen (TN)	% of TS	1.5 – 4	1.6 – 6.0	2.4 – 5.0	3.5
Total phosphorus	% of TS	0.8 – 2.8	1.5 – 4.0	2.8 – 11.0	2.8
Potassium	% of TS	0 – 1	0.0 – 3.0	0.5 – 0.7	0.2
Cellulose	% of TS	8.0 – 15.0	8.0 – 15.0	nd	nd
Iron	% of TS	2.0 – 4.0	3.0 – 8.0	nd	nd
Silica	% of TS	15.0 – 20.0	10.0 – 20.0	nd	nd
Alkalinity	mg$CaCO_3$/L	500 – 1500	2500 – 3500	580 – 1100	nd
Organic acids	mg acetic acids/L	200 – 2000	100 – 600	1100 – 1700	nd
pH		5.0 – 8.0	6.5 – 7.5	6.5 – 8.0	nd

nd = no data

Nitrogen exists in sludge in either inorganic forms such as an ammonium (NH_4^+) or nitrate or a complex organic form. The concentrations of both inorganic and organic nitrogen are dependent on the types of sludge and handling processes. In comparison to organic nitrogen, inorganic nitrogen is reduced more easily by dewatering and drying procedures. Some insoluble sludge constituents like phosphorous or calcium remain in sludge, and are further decreased during dewatering. In contrast, potassium and sodium, soluble constituents, will follow treated wastewater [10].

Numerous specific organic chemical contaminants are present in sewage sludge. Of these compounds, trace organic contaminants such as pharmaceuticals and endocrine disrupting chemicals (EDCs) are of concern since their applications may results in greater risks to human and environment [32]. In a recent literature review, Harrison et al. [40] examined available data on the presence of organic chemicals in sludge in US. Data were reported to 516 potential organic pollutants, which were grouped into 15 classes and their range of concentration was also recorded.

Raw sewage sludge, which does not experience any treatment, is regarded as a source of pathogens, including bacteria, viruses, fungi, yeast, and parasitic microorganisms. They present a public health hazard, so their quantity should be figured out. In reality, the quantity and types of pathogens vary depending on the health status of a community.

2.2 Anaerobic sludge digestion

2.2.1 Fundamentals of anaerobic digestion

AD is a process in which organic matter can be biodegraded in the absence of oxygen by a consortium of microorganisms. An important product of AD is biogas, which mainly comprises CH_4, carbon dioxide (CO_2) and traces of other gases [3]. AD involved a series of biochemical reactions, which can be divided into four stages, namely hydrolysis, acidogenesis, acetogenesis and methanogenesis (Figure 3). AD has been extensively used to treat biodegradable organics and produce biogas [5].

AD is a sequential process involving several complex biochemical stages. Each stage is consistently performed under activities of a specific group of bacteria or interactions of different ones. In the hydrolysis step, hydrolytic microorganisms hydrolyse polymer materials to form monomers, such as amino acids and glucose. These monomers are subsequently converted into H_2, CO_2 and short-chain fatty acids such as acetic, propionic, and butyric acids in the acidogenesis step. In the acetogenesisstep, syntrophicacetogenic bacteria metabolize these volatile fatty acids (VFAs) to produce precursors for the methanogenic fermentation. Finally, CH_4 is formed from either acetate or CO_2 and H_2 by methanogenic bacteria in the methanogenesis step [4, 41, 42].

2.2.2 Current status of the anaerobic digestion process

Anaerobic conversion is a natural process occurring in various environments, such as wetlands, rice fields, intestinal tracts of animals, marine or fresh water sediments. Humans have harnessed this process to take benefits as energy, rapid decomposition of organic waste, and stabilised residue for a long time. Historically, applications of AD could be dated back to the 10^{th} century, when it was used to produce combustible gases from sediments in lakes, ponds, and streams. The very first design for AD with the full-scale application was conducted for domestic wastewater treatment in the

18th century. The recovery of biogas from well-designed sewage treatment facilities was established in 1895 in England, treating 230 m^3/d of wastewater; and the collected gas was used to fuel street lamps [41, 43].

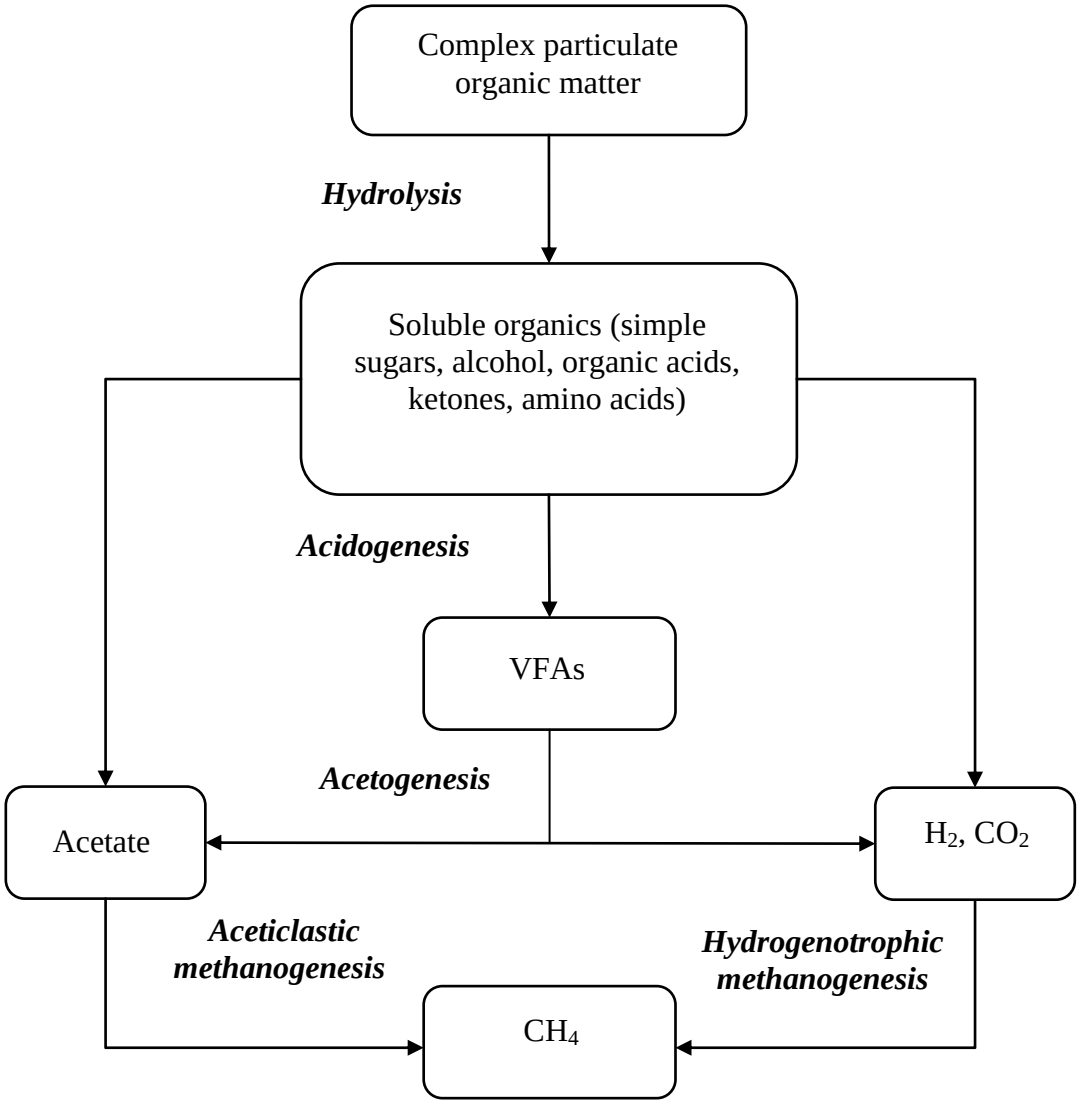


Figure 3. Different stages of AD process (modified from [4, 41, 42])**.**

Over the last two decades, a great deal of the literature has been published on feasible applications of AD for solid waste and wastewater treatment. Apart from biogas production, AD proves much greater potential due to more intrinsic merits, including energy saving, nutrient recovery, reduction of waste organic content and pathogen populations as opposed to the conventional aerobic digestion [4-6]. As a result, extensive applications of AD have been only revealed very recently with a number of developing designs by focusing on more complicated devices and operational techniques, and increased understandings of microbiology and biochemistry. There are various anaerobic reactor types in practice, of which batch

reactors are the simplest configuration. The one-stage continuously fed systems, the two-stage and multistage continuously fed systems were more advanced reactors applied for AD treatment [44].

The evolution of AD applications was also confirmed by a broad range of potential substrates for this process. Anaerobic technology such as single-phase (conventional) and two-phase anaerobic digesters was often used in the treatment of dairy wastewater for high energy production and waste stabilization. In terms of low content of suspended solids in dairy wastewater, the conventional anaerobic reactors are generally nominated for treatment. Currently, numerous studies of dairy wastewater treatment have shown a wide range of applicable anaerobic reactor designs, including downflow fixed film, anaerobic filter, up-flow anaerobic sludge blanket (UASB), hybrid UASB, anaerobic sequencing batch reactors (ASBR), ASBR upflow packed-bed, rotating biological contact reactor, upflow anaerobic solid removal reactor, multichamber bioreactor, continuously stirred tank reactor (CSTR). At laboratory scale, the efficient removal of chemical oxygen demand (COD) of these reactors could reach up more than 90% [6].The authors also reviewed the two-phase anaerobic treatment, which was employed for dairy wastewater consisting of high concentrations of non-filtered solids and lipids. The dominant reactor type was CSTR and upflow filter, with CSTR used for acidogenesis stage and upflow filter responsible for methanogenesis. Compared to conventional AD processes, the two-phase shows better outcomes with various kinds of industrial wastewater. Sludge produced from WWTPs has also aroused much consideration since some strict rules of sludge disposals were adopted [41]. Due to advantages of AD, it has become one of the promising solutions for sludge stabilisation and energy production [30, 45, 46]. Current improvements of high-rate anaerobic system have been drawing more attention on AD performances in agricultural waste treatment, especially animal residues [44] which have different characteristics from those of municipal and industrial wastewater [7]. Anaerobic treatment of the poultry and livestock manure waste, two other types of agricultural waste, were also of interest due to increasing concerns of their disposal [47-49], there has been having more and more investigations of the AD process on them. The type of reactors used for livestock manure waste treatment include: batch, continuous one stage, and continuous two stage reactors, tubular reactor, ASBR, AF or the plug flow reactor (PFR) as

described in Table 2. Many studies have also indicated that co-digestion of poultry and swine manures with each other or with additional substrates like sewage sludge, some industrial byproducts proves more beneficial in biogas production and even more efficient in substrate treatment [5, 44, 47, 50].

2.2.3 Factors influencing the anaerobic digestion process

AD can be sensitive to several operating factors, including pH, alkalinity, temperature, and characteristics of the substrates (Figure 4). To optimise the efficiency of AD, these factors should be carefully regulated.

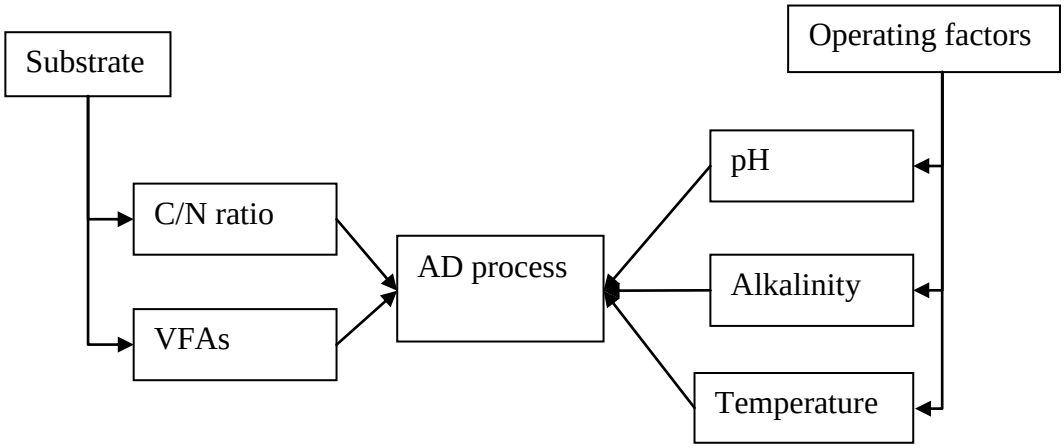


Figure 4. Factors influencing AD performance.

2.2.3.1 pH

pH fluctuation can influence biogas yield throughout AD. In the early stages (i.e. hydrolysis, acidogenesis, and acetogenesis), pH decreases due to the formation of organic acids. Once the methanogenesis step occurs, pH may increase slightly because of the production of ammonia [42]. Below pH 6, inhibition of CH_4-forming bacteria can occur and the anaerobic process can be disrupted [8]. The pH inside digesters is an important factor governing the growth of anaerobic microbes, particularly methanogens, through its impact on enzyme activities. This is because each group of microorganisms has its own appropriate pH for growth. Methanogenic bacteria are more sensitive to pH and need a pH range between 6.5 and 7.8 [47] while acid-forming bacteria can function in a wider pH range from 4.0 to 8.5 [51] but prefer a pH of 5.5 to 6.5 [44, 52]. In operation, it is necessary to keep the pH close to neutral since methanogenesis is the yield-limiting step. Lime addition is a common technique to overcome pH reduction.

Table 2. Operational data of different anaerobic bioreactors used for different types of agricultural waste [5, 44, 47, 50].

Agricultural waste	Reactor configuration	Reactor volume	OLR	HRT (days)	Temperature (°C)	COD or VS removal	Biogas or CH_4 yield
Poultry manure	Batch reactor, UASB, pilot and full-scale digesters	0.1 L – 95 m^3	1.1 – 2.9 gCOD/L.d	0.5 – 305	25 – 55	32 – 78% COD	31 – 548 $mLCH_4$/gVS 3.6 – 368 mLbiogas/gVS
Cattle manure	Fixed-film reactor, attached-film bioreactor, anaerobic rotating biological reactor, batch reactors, downflow anaerobic filter, fixed dome plant, UASB, CSTR, UAF, TPAD, AHR, and two-stage anaerobic systems	120 mL - 1300 m^3	0.117 – 7.3 gVS/L.d	0.5 – 140	5 – 82	37.9 - 94% COD 7.3 - 92% VS	93 – 382 $mLCH_4$/gVS 103 – 450 mLbiogas/gVS
Swine manure	Hybrid UASB, CSTR, baffled, ASBR, batch reactor, dispersed growth, stirred batch, and PFR	125 mL – 565 L	0.9 – 15.4 gVS/L.d	0.9 – 113	22.6 – 60	57 – 78% COD	22 – 360 $mLCH_4$/gVS 207 – 249 mLbiogas/gVS

Organic loading rate (OLR); hydraulic retention time (HRT); up-flow anaerobic filter (UAF); continuously stirred tank reactor (CSTR); anaerobic sequencing batch reactor (ASBR); anaerobic hybrid reactor (AHR); temperature-phased AD (TPAD); plug flow reactor (PFR)

2.2.3.2 Alkalinity

Alkalinity refers to the buffering capacity, which is important for regulating pH in AD. Alkalinity originates from the degradation of organics in the form of CO_2, bicarbonate and ammonia [51]. The equilibrium of CO_2 and bicarbonate will resist the rapid changes in pH. Compared to pH, alkalinity or buffering capacity gives more reliability for system stability since the possible accumulation of VFAs can lead to a reduction in buffering capacity and pH [53].

The pH in an anaerobic system is controlled by CO_2 in the gas phase and bicarbonate in the liquid phase. Thereby, pH will increase when more bicarbonate is added. In practice, when the digester pH decreases a net strong base, either sodium hydroxide or calcium hydroxide [47] or carbonate salts, are utilised. They are able to remove CO_2 in the gas phase to convert into bicarbonate. Bicarbonate can be directly added to eliminate the lag time and over organic dosing [44].

2.2.3.3 Temperature

AD strongly depends on temperature because not only does temperature affect the physicochemical properties of substrates in digesters, but bacteria are also sensitive to any changes in temperature. Therefore, it is essential to maintain constant favourable temperatures for the growth of anaerobic microbes [8]. Insulation, water baths or passive solar heating are used for temperature maintenance; and heat can be added by using heat exchanges in the recycled slurry or heating coils or steam injection in the digester [13]. Any fluctuation of temperature even small change from 30 to 32 °C [44], would lead to inactivation of bacteria, resulting in a decrease in biogas production. Moreover, process failure can be observed at temperature changes in excess of 1 °C per day [54].

There are three temperature ranges investigated for applications: psychrophilic temperature from 10 to 20 °C, mesophilic temperature from 20 to 40 °C, and thermophilic temperature between 40 and 60 °C [47]. Once sufficient retention time for the CH_4-producing bacteria is provided, anaerobic sludge digestion could be operated successfully at psychrophilic temperature as low as 20 °C [44]. The main discrepancy between mesophilic and thermophilic digestion lies in the CH_4 yield. It is documented that higher CH_4 produced by thermophilic digestion compared to that by mesophilic digestion in a given digester due to the fact that high temperature is

the preferred condition for methanogens growth [8, 55]. Another advantage of thermophilic digestion is pathogen removal since certain pathogens could be killed at high temperature. Moreover, thermophilic conditions were evaluated to facilitate the balanced fermentation system in biogas production by [56]. The application of high temperature, however, has some drawbacks, such as increase in free ammonia or VFAs, which make the process more susceptible to inhibitors [54].

2.2.3.4 VFAs

VFAs created during AD are an important intermediate product and relates to the imbalance of AD. High VFA concentration primarily causes the process failures with respect to an imbalance among acidogenic, acetogenic and methanogenic organisms [14]. Limiting concentration of VFAs for a stable performance of a anaerobic digester was reported at13000 mg/L by Viéitez et al. [57]. Additionally, less effective removal of COD is observed with increased VFA production [47]. In the acetogenic stage, the VFA accumulation will lead to a decrease in pH, which directly inhibits the growth of methanogens. If inhibition lasts in long time, acetogens will predominate in digesters. As discussed, the addition of buffering is an effective solution since this can resist pH drop and maintain sufficient VFA concentration for subsequent reactions [44]. While acetic acid is the key substrate for methanogenesis, it is determined that propionic and butyric acids are inhibitory to methanogenic bacteria. So as to avoid digester failures, monitoring of VFA, especially butyric acids, has been shown to stabilise the overall system [44].

2.2.3.5 Ammonia

The present of ammonia in digester results from the breakdown of nitrogen-containing matter, mainly from protein and urea. Ammonia is regarded as one of inhibitory substances to the AD process [58]. Between two forms of ammonia, NH_4^+ and free ammonia (NH_3) in liquid, free ammonia has been identified as the main cause of inhibition. The reason is the hydrophobic form of ammonia could easily penetrate through cell walls, causing pH imbalance and enzyme malfunction [58]. This inhibition in general has been clearly observed in the methanogenesis stage. Koster and Lettinga showed that along with the increase of ammonia concentrations in the range of 4051 – 5734 mgNH_3–N/L, acidogenic populations in the granular sludge were hardly affected while the methanogenic population lost 56.5% of its

activity [59]. On basis of CH_4 production, ammonia has stronger effect on aceticlastic than hydrogenotrophic methanogens. It is suggested that the free ammonia should be kept below 80 mg/L, while ammonium could reach up to 1500 mg/L without causing any inhibition [55]. It is pH and temperature that become factors influencing the ammonia inhibition capacity through ammonia concentrations [58]. The more pH increases, the more the amount of ammonia and less the amount of ammonium are.

2.2.3.6 C/N ratio

The C/N ratio is a common parameter that has been thoroughly investigated by numerous studies of AD. The C/N ratio represents the relative amount of organic carbon measured by COD and nitrogen present in feedstock. The changes in the specific CH_4 yields and CH_4 content consistently comply with the C/N ratio. Low nitrogen content would lead to the inhibition of AD since anaerobic microbes needs an adequate amount of nitrogen for their growth while organic carbon is considered as a sole source for anaerobic activity. Increase in pH can result from low C/N ratio. On the other hand, a high C/N ratio would directly result in a rapid conversion of nitrogen and low biogas production. It has been elucidated that the ideal levels of C/N ratio should be in the range of 20 – 30, typically approximately 25 [13]. For instance, the peak values of CH_4 yields were observed at a C/N ratio of 23 [60].

2.2.4 Anaerobic digestion in sewage sludge treatment

Through wastewater treatment systems, only liquid can be disposed of to the environment while solids are collected for further treatment before discharge, of which, sludge is by far the largest component in volume. Sludge is considered as both a potential pollutant and a prospective source. Accordingly, sludge treatment and disposal should be always an integrated part of wastewater treatment. Therefore, it is imperative to seek environmentally sound and sustainable methods for sewage sludge handling and disposal. Sustainable sludge treatment may be defined as “a method that meets requirements of efficient recycling of resources without supply of harmful substances to human or environment” [2]. Thereby, not only sludge management but also research into innovative handling methods should concentrate on three main objectives: (1) complete sludge treatment with regards to reduction of sludge levels, (2) recovery of valuable products from sludge, regarding the nutrient

used as a soil conditioner or improver, and biogas production, and (3) choice of a treatment method at an acceptable cost [28, 31].

In terms of disposal of excess sludge, the 1990 North Sea Conference consented to an agreement of banning of the sludge dumping at sea and instead using biosolids in agriculture. There is a need to find replacements for the dumping of sludge at sea. The possible fate of excess sludge are landfill, incineration, spray irrigation, drying, composting, and AD. They are towards the final goal – the transformation of wastewater sludge into the innocuous and easily dewatered form. Several studies [54, 55, 61] have confirmed that AD is an ideal method compared to other methods of sludge destabilisation based on its merits as shown in Table 3. This table also lists some drawbacks of the anaerobic sludge digestion, which greatly demands consideration.

Table 3. Advantages and disadvantages of anaerobic sludge digestion [5, 6, 10, 52, 62].

Advantages
- Production of CH_4, a renewable source of energy, compensating energy for maintaining the temperature of digesting sludge, and meeting requirements for mixing; additionally, heat buildings, drive engines of aeration blowers or generate electricity that can be used to run the sewage pumps - Net reduction of mass and volume of sewage sludge during the conversion of organics into CH_4 and CO_2 gas and water - Transformation of solid content to stable, inoculum sludge - Beneficial reuse in agriculture – soil conditioner or fertilizers: treated sludge may contain N, P and other nutrients and organics can improve soil quality - Inactivation of pathogens during AD
Disadvantages
- The high capital costs: large, covered tank as well as pumps for feeding and circulating sludge, heat exchangers and compressor for gas mixing - Long HRT necessary to develop and maintain the population of CH_4-producing bacteria - The quality characteristics of supernatant from anaerobic sludge digestion are poor, containing suspended solids, dissolved and particulate materials, nitrogen and phosphorus

2.3 Anaerobic co-digestion of sewage sludge with other substrates

The efficiency of AD can be determined as the volume of produced biogas or the amount of substrate depletion or the formation of intermediates and other final products during. These performance indicators are inter-related, thus, it is generally reliable to assess the performance of AD based on the biogas yield. The more efficient the anaerobic treatment, the more biogas/CH_4 production will be generated. Biogas produced from AD facilitates a sustainable development of energy supply both economically and environmentally. As compared to the energy yield of aerobic degradation, anaerobic biomass conversion results in low energy, because which is mainly stored in biogas. This energy is subsequently harvested in the presence of oxygen by aerobic organisms or by humans through heating and other processes [63].

2.3.1 Advantages of anaerobic co-digestion in treating sewage sludge

With the aim of improving biogas quality and quantity, certain possible approaches, including process design improvement, pretreatment of substrates, removal of toxic components and co-digestion should be considered. Improving process design could be performed via either increasing biomass retention since a dense mixture of microorganisms is essential for the biochemical metabolism of complex substrates, or advancing configuration and operation in terms of temperature and HRT. As an obvious example, enhanced biogas production levels and COD/VS removal efficiency of manure along with a series of bioreactor designs were reported (Table 2). Regarding sludge, Athanasoulia et al. [57] compared biogas production potentials between a cascade of two methanogenic CSTR in a series, and a conventional one-step CSTR reactor treating sewage sludge, concluding that the biogas production was considerably improved by 9.5% to 40.1% in the serial digestion. In terms of VFAs, values were higher in the cascade configuration, from 31.5% to 33.8%, in comparison with the one-step process, from 36.2% to 40.7%. Another way to increase biodegradability is the pretreatment of substrates which are hardly hydrolysed with a high content of cellulose and hemicellulose [64]. Mechanical destruction, heat treatment, and chemical treatment are pretreatment that techniques have been extensively applied for domestic sludge [14, 46, 65, 66]. The removal of toxic or inhibitory compounds in the feed prior to AD, contribute to increase biogas yield. Ammonia levels can be decreased by free-ammonia stripping or bentonite

bound oil while ferric chloride and ferrous chloride pose their effect on precipitating sulphate/sulphide [14, 67]. Up to now, results from the literature have clearly demonstrated that the most successful way in improving biogas yield should be co-digestion [68-71]. This can be explained by certain benefits of co-digestion: (1) dilution of potential toxic compounds, (2) the supply of missing nutrients, (3) synergistic effects of microorganisms, (4) increased load of biodegradable organic matter, (5) economic advantage of sharing equipment, and (6) better biogas yield [16, 17]. AD has mainly taken place for the treatment of sewage sludge or other wastes. Recently, for instance, manure from pigs, cows, and chicken is currently digested with co-substrates, which include harvest residues, organic wastes, food wastes, municipal bio-wastes, and energy crop for higher biogas production [8, 9].

2.3.2 Current status of anaerobic co-digestion of sewage sludge and other organic materials

Although co-digestion could offer many benefits [69, 72], the application of co-digestion of sewage sludge and other organic materials is still not well understood. In recent years, much successful efforts have been made for such co-digestion in order to upgrade the role of anaerobic degradation in stabilising sewage sludge and produce feasible bioenergy as well [73].

The quality and composition of biogas produced could not be significantly changed by OLR, HRT or other operational parameters but considerably depend on their origin and substrate compositions, such as carbohydrates, protein, and lipids [74], whose concentrations in sewage sludge were documented to be varied from one type of sludge to another one (Table 1). Among them, lipids has been known as a very promising substrate with regards of the CH_4 production (i.e. 1014 $mLCH_4/gVS$), but requires more time for complete biodegradation. Meanwhile, proteins and carbohydrates show faster conversion rate but lower biogas levels, i.e. 496 $mLCH_4/gVS$ and 415 $mLCH_4/gVS$, respectively [8, 64, 75]. Through investigations on 175 BMP assays, Labatut et al. [76] revealed that substrates rich in lipids and easily biodegradable carbohydrates yield the highestCH_4 potential, while others are more recalcitrant with a high lignocellulosic fraction and pose the lowest gas production rates. This could explain why hydrolysis has been reported as the rate-limiting step in the AD of sewage sludge since proteins have been reported as the

rich composition in sewage sludge rather than carbohydrates and lipids. Therefore, the addition of easily hydrolysed substrates to sewage sludge during AD must initially accelerate the growth of microorganisms, speeding up the whole process as a result. One of the key operating parameters of AD process, the C/N ratio, is another concern related to sewage sludge. As discussed above, due to the low C/N, around 6 – 9, co-digestion of sewage sludge with high carbon content substrates is a necessity for better anaerobic degradation and higher biogas production.

Taking the biogas production potential into account, any type of biomass containing carbohydrates, proteins, fats, cellulose, and hemicelluloses as main components could be typical substrates for AD [77]. As such, potential co-substrates for AD should be categorised into harvest residues, including top and leaves of sugar beets, organic wastes, food wastes, municipal biowaste from households, energy crop, and sewage sludge [8, 9]. Table 2 summarises the biogas/CH_4 production potential and VS removal ability when mixing sewage sludge with some different promising substrates, including fats, oil and grease (FOG), animal byproducts from the meat-processing industry, and coffee waste, organic fraction from municipal solid wastes (OFMSW), brewery sludge, manure, and glycerol. Anaerobic co-digestion has been mainly performed at mesophilic temperature range, from 35 °C to 37 °C, with various bioreactor systems. Operating at thermophilic temperature lowered the CH_4 yield but enhanced VS removal. It is clear that the addition of co-substrates makes AD achieve greater biogas production. The current studies have shown the differences in biogas percentages in a mixture of biogas. In comparison, the highest CH_4 content was observed in the gas coming from the sewage digester, from 57.8% to 65%, while the lowest CH_4 and highest nitrogen content were found in the landfill gas, from 37% to 62% [78-81]. From Table 4 of typical concentrations of biogases, it can be seen that CH_4 and CO_2 are the predominant components resulting from anaerobic degradation. Unlike CO_2 which is more water soluble, CH_4 is partially non-soluble and takes up for the most part in gas phase [68]. Therefore, it might be more reliable to assess CH_4 potential rather than biogas potential.

Each co-substrate has its own CH_4 production capacity. Sewage sludge, typically primary sludge and waste activated sludge [70, 72], contains certain easily degradable material [16]; and its CH_4 production potential could be approximately 300 – 400 $mLCH_4/gVS_{added}$ [69, 71].

Table 4. Biogas composition [9].

Combustible ingredients	Concentration (%)
CH_4	50 – 70
H_2	< 1
H_2S	2
Non-combustible ingredients	**Concentrations (%)**
CO_2	25 – 50
H_2O	2 – 7
O_2	0 – 0.5
NH_3	0 – 2

As mentioned above, lipids are the most attractive macromolecular component during the anaerobic transformation for its high theoretical CH_4 production. Luostarinen et al. [82] reported that the CH_4 yield potential of grease trap sludge (GTW) is 918 $mLCH_4/gVS_{added}$. The chemical and physical properties of GTW could differ greatly depending on origin and grease abatement device (Table 5). Several studies on this process observed an increase of 30% in biogas production and a recovery of more than 50% in energy for on-site generation when directly adding FOG, the top floatable layer of GTW, from the food industry [83, 84]. Although FOG fraction in GTW is low, at average value of around 2 – 3% by volume [85], it has been considered a potential feedstock for both AD and biodiesel production processes due to its high fraction of lipids [72, 86]. However, lower pretreatment expenses during the anaerobic FOG conversion makes it a more feasible option of disposal compared to the biodiesel production [85].

In recent years, despite enhanced CH_4 potential has been achieved by anaerobic co-digestion of FOG and sewage sludge, several operational concerns of the inhibition of methanogens due to FOG and its derivations have been stated. The detrimental effects of FOG on methanogenic bacteria greatly attributed to the high content of long chain fatty acids (LCFAs). There are three main mechanisms, including the inhibition of aceticlastic and hydrogenotrophic methanogens due to the LCFAs toxicity, the sludge flotation and biomass washout due to the adsorption of LCFAs onto sludge, and the movement limitation of bacteria when its cell wall is covered by a LCFAs layer [85-87]. Therefore, the AD process of FOG starts slowly. The CH_4 production of FOG was only improved when FOG is mixed with sewage sludge. The higher CH_4 yield achieved from this digestion is illustrated in Table 6.

OFMSW is also a suitable co-substrate since it is the easily degradable content of up to 40% in municipal solid wastes organic fraction, accounting for more than 40% of municipal solid wastes (MSW) [88], is the easily degradable constituent. Concerns related to the OFMSW management via conventional methods, include composting, incineration, landfilling, and dumping [56]. Accordingly, anaerobic co-digestion of OFMSW and sewage sludge becomes one valuable alternative to these approaches in terms of energy recovery and environment security. Differences in characteristics of OFMSW are caused by various sources. In one study carried out by Cabbai et al. [89], source selected OFMSW from canteens, bakery, restaurants, supermarkets, and households vary in compositions (Table 5). Compared to sewage sludge, OFMSW has a higher C/N ratio and lipid load that elevate the initial stage of AD, which may benefit CH_4 production in case of co-digestion [88]. Significantly, higher CH_4 productivity during co-digestion was observed under various conditions compared to the single sludge anaerobic conversion (Table 6). Among several OFMSWs, food waste in general is also considered the most variable substrate depending on its origin, such as household, restaurants, markets, university dining hall, and components, in terms of carbohydrates, proteins, and lipids [78]. Food waste diversity in characteristics is also expressed in element compositions, in which sufficient carbon content makes food waste the highly degradable substrates in AD. In general, food waste has high VS/TS ratio (80 – 90%) and moisture content (75 – 85%), which makes landfill applications of food waste inadequate due to environmental issues, namely odour release, toxic gas emission, and groundwater contamination [90].

Instant coffee waste characteristics are mainly dependent on the production procedure applied and raw matter used. In general, coffee waste mainly contains carbohydrate in forms of fibres, namely lignin, cellulose, and hemi-cellulose regardless of original materials used [91, 92]. On one hand, it has been reported from the literature that AD of coffee waste was implemented at both mesophilic [92] and thermophilic temperatures [35] for such high carbon content. These investigations, on the other hand, indicated that the high concentration of solids and fibre could lead to some problems such as clogging, but still yield the high CH_4 composition of 70%. This makes necessary to evaluate the AD performance of each kind of waste in an anaerobic co-digestion with other substrates. The feasibility of anaerobic treatment of

coffee and sewage sludge was assessed, and resulting CH_4 yield together with VS reduction were shown relatively high (Table 6). The low CH_4 yield of manure is attributed to the high water content as well as high content of fibre, typical ranging from 10 to 20 m^3 of CH_4 per tonne of treated manure [93].

Table 5. Characteristics of certain co-substrates used for anaerobic digesting with sewage sludge.

Types of co-substrates		Crude glycerol	FOG	OFMSW
Characteristics	TS (%)	nd	1.8 – 97.2	8 – 70
	VS/TS (%)	nd	88.9 – 98.6	84.1 – 98.0
	COD (g/L)	1, 054 – 1,216	nd	nd
	pH	5.0 ± 0.1	nd	3.7 – 4.6
	Density (kg/L)	1.25 ± 0.1	nd	nd
	Electrical conductivity (μS/cm)	4.2 ± 0.3	nd	nd
	TN (% of TS)	nd	nd	1.7 – 3.3
	C/N	nd	nd	13.7 – 31.4
Components (% w/w)	Carbohydrates	nd	0.5 – 15	10.7 – 42.5
	Protein	nd	0.3 – 7	9.7 – 20.5
	Fat	nd	78 – 99.5	5.8 – 25.0
	Glycerol	50 – 60	nd	nd
	Alkalis	12 – 16	nd	nd
	Methyl esters	15 – 18	nd	nd
	Methanol	8 – 12	nd	nd
References		[18-20]	[85]	[89]

nd = no data

Considered as a non-toxic, affordable, renewable and environmentally friendly alternative to petroleum-derived fuels, biofuels, namely biodiesel and bioethanol, have become the greatly demanded energy source. This led to the exponentially increasing production of biofuels along with a surplus of crude glycerol since glycerol is an inevitable co-product from not only biodiesel chemically and enzymatically manufacturing [94], but bioethanol production also [95, 96]. Crude glycerol is the main co-product in the biodiesel production. The ratio of produced crude glycerol and biodiesel was estimated at 0.1 [18]. Consequently, such extra crude glycerol has generated both a reduction in its market price, ten times as reported recently by Yazdani et al. [95] and an environmental issue due to the disposal of waste stream containing glycerol [18] as well as polluted wastewater

from purification processes [20]. There was a need of effective treatment processes to transfer crude glycerol to higher valuable products that could improve economic feasibility of biofuel industry. CH_4 production by the anaerobic fermentation of glycerol offers a great chance for energy supplementation in biofuel industry. It is the high carbon content of crude glycerol that makes it an ideal substrate for anaerobic conversion when the high production of biogas is taken into consideration. Advantages of crude glycerol are the highly solubility since major components of are pure glycerol, alkalis, methyl esters, and methanol as given in Table 5, as well as the easy storage capacity of glycerol over a long time. It was reported that a large range but low concentration of impurities, especially elements, caused by glycerol purification processes and different used feedstocks have not exerted any significant inhibitions to AD. The high salinity, sodium and potassium salts of crude glycerol could have a negative effect on microbial activity [18]. The failure of the anaerobic degradation of crude glycerol possibly results from the low concentration of N, which is an essential element for microorganisms. However, the co-digestion with sewage sludge completely turns it beneficial due to low C/N of sewage sludge. Until now, little research work has been published regarding such co-metabolism. When treating sewage sludge from WWTPs, Fountoulakis et al. [19] investigated that the supplementation of glycerol can increase biogas production (Table 6) at concentration of 1% by volume. Imbalance of the system was observed when adding higher glycerol concentrations.

Table 6. Co-digestion of sewage sludge with different substrates.

Feedstock	Bioreactor system	Temperature (°C)	CH_4/Biogas production	CH_4/biogas increase (%)	VS removal (%)	References
SS + FOG (52 : 48)	Semi-continuous digester	35	449 $mLCH_4STP/gVS_{added}$	195	45	[84]
		52	512 $mLCH_4STP/gVS_{added}$	160	51.2	
SS		35	159 $mLCH_4STP/gVS_{added}$	-	25.2	
		52	197 $mLCH_4STP/gVS_{added}$	-	30.7	
SS + GS (90 : 10)	Continuous-pilot scale digester	35	295 $NmLCH_4/gVS_{added}$	9	45 – 58	[37]
SS + GS (70 : 30)			344 $NmLCH_4/gVS_{added}$	27		
SS			271 $NmLCH_4/gVS_{added}$	-	nd	
SS + GS (54 : 46)	Lab-scale digester	35	463 ± 48 $mLCH_4/gVS_{added}$	66.5	59 – 70	[82]
SS			278 $mLCH_4/gVS_{added}$	-	nd	
SS + FOG (80 : 20)	Lab-scale single stage digester	35	510 $mLbiogas/gVS_{added}$	18	57	[38]
SS + FOG (40 : 60)			640 $mLbiogas/gVS_{added}$	50	59	
SS			430 $mLbiogas/gVS_{added}$	-	nd	
SS + OFMSW (50 : 50)	Fed-batch digester	35	360 $mLCH_4/gVS_{added}$	93.5	57	[56]
		55	180 $mLCH_4/gVS_{added}$	45	64	
SS		35	186 $mLCH_4/gVS_{added}$	-	26.6	
		55	124 $mLCH_4/gVS_{added}$	-	nd	

Feedstock	Bioreactor system	Temperature (°C)	CH_4/Biogas production	CH_4/biogas increase (%)	VS removal (%)	References
AS + Coffee waste	Dual digestion system	35, 55	310 $mLCH_4/gVS$	nd	41 – 52	[97]
	Single stage digester	35	280 $mLCH_4/gVS$	nd	28 – 50	
AS + Coffee waste	Batch assay	37	240 – 280 $mLCH_4STP/gVS_{added}$	nd	75 - 80	[91]
SS + ABP	Lab-scale digester	35	400 ± 30 $mLCH_4/gVS$	nd	nd	[98]
SS + ABP			410 ± 30 $mLCH_4/gVS$	nd	nd	
SS + brewery (75 : 25)	Bench-scale CSTR	36 ± 0.2	510 $mLbiogas/gVS_{removal}$	27.5	16.4	[99]
SS + brewery (50 : 50)			610 $mLbiogas/gVS_{removal}$	52.5	18.2	
SS + brewery (25 : 75)			650 $mLbiogas/gVS_{removal}$	62.5	19.0	
SS			400 $mLbiogas/gVS_{removal}$	-	14.9	
SS + FW +CM (10 : 20 : 70)	Continuously stirred-tank reactors	36	603 $mLCH_4/gVS_{added}$	nd	nd	[100]
SS + Gly (99 : 1)	CSTR	35	2353 ± 94 $mLCH_4/d$	112.7	nd	[19]
SS			1106 ± 36 $mLCH_4/d$	-	nd	

Standard temperature (0 °C) and pressure (1 atm) (STP); normal mL at 1 °C and 1 atm (NmL); sewage sludge (SS); grease trap sludge (GS); activated sludge (AS); animal byproducts (ABP); food waste (FW); glycerol (Gly); and cattle manure (CM); nd = no data

2.4 The behaviour of trace organic contaminants during the anaerobic digestion process

Concerns about the occurrence of TrOCs in wastewater and sewage sludge have increased in recent years. It have been widely reported that their concentration levels, removal rates as well as their behaviour significantly differ throughout wastewater treatments. This could be explained by a variety of applied treatment processes, operational conditions, and other factors such as temperature, pH and seasonality [21, 22]. During wastewater treatment, TrOCs will appear in the supernatant to be recycled in outlet and partly accumulate in sewage sludge [22, 25]. Carballa et al. [25] conducted a comprehensive study on the behaviour of pharmaceutical and personal care products (PPCPs) during wastewater treatment and detected most of PPCPs, concluding that they were present in both the primary and secondary sludge fed to anaerobic digesters. Samaras et al. [21] determined that the removal of such TrOCs as nonylphenol and triclosan was attributed to the absorption mechanism onto sludge while biodegradation/biotransformation was the significant mechanism for contaminants such as ibuprofen, diclofenac, and ketoprofen.

2.4.1 Trace organic compounds and their effects

Although the classification of TrOCs has not been fully published, these compounds could be simply divided into certain main categories, including pesticides, pharmaceutically active compounds, and EDCs. This classification mainly depends on their particular intended usage. That means their source might correlate to where they have been employed. Through several routes, TrOCs could contaminate natural water bodies, such as lakes, rivers and underground streams. Numerous documents have reported the potential effects of TrOCs on human health, aquatic biota, and environment [101]. Plants and animals could take up some anthropogenic organic chemicals inside sludge, which might accumulate in the food chain. As such, some of them potentially have severe effects on flora and fauna and soil as well. These effects pertain to blocking or hampering functions of hormones in human and animals [101], feminisation of male fishes by EDCs [102], estrogenic effects in rates caused by bisphenol A [103].

2.4.2 Occurrence of emerging contaminants in sludge

Concentrations of TrOCs range between few ng/L to some g/L in wastewater, surface water, groundwater and drinking water. While in sewage sludge from WWTPs, their concentrations could reach up to a few mg/kg [21].

Differences in concentration are observed among different groups of compounds as well as different countries and WWTPs. This is because their concentrations in sludge widely depend on various factors. Concentrations of original compounds in influent wastewater directly govern their final concentrations in sludge. Another factor is their physicochemical properties, such as solubility and molecular weight. Sludge characteristics and operational conditions applied in WWTPs also have great effects on occurrence of TrOCs in sludge [104, 105].

The abundance of TrOCs in sludge has been reported at different levels. In general, sludge is dominated with surfactants, of which linear alkyl benzene sulphonates (LAS) has the highest concentrations in sludge due to their wide usage. Other two surfactants taken into account are nonylphenolethoxylates and quaternary ammonium-based compounds. Concentrations from few mg/kg to more than g/kg has been reported as a range of surfactants in sludge [106]. Regarding PPCPs, the highest concentrations in sludge belong to hydrophobic compounds, including triclosan, triclocarban, and galaxolide. Their range of concentration vary from some μg/kg to mg/kg [107]. Some TrOCs from industrial applications, brominated flame retardants, organotins, and perfluorinated compounds have been detected at lower concentrations, few μg/kg [108-110] in sludge compared to phthalic acid esters, in the range of some mg/kg [111] while limited data have reported in the presence of nanoparticles and benzothiazoles.

In the sector of agriculture, several guidelines stated the limiting concentrations of these compounds in biosolids management. Accordingly, their concentrations in sludge vary depending on a particular contaminant. EU, for instance, proposed their acceptable levels in sludge prior to agricultural reuse as shown in Table 7.

Table 7. Proposed limit values of TrOCs in sewage sludge [112-114].

No	TrOCs	Limiting concentrations in sludge prior to reuse (μg/g)
1	Linear alkylbenzenesulfonates	2600
2	Diethylhexylphthalates	100
3	Nonylphenolethoxylates	50
4	Dioxins and furans	0.0001
5	Organohalogens	500
6	Polychlorobiphenyls	0.8
7	Polycyclic aromatic hydrocarbons	6

2.4.3 Anaerobic digestion in the removal of trace organic compounds

The appearance of TrOCs in sewage sludge has been widely reported in the literature. Their concentrations considerably differ among different groups of compounds, ranging from some μg/kg to several g/kg [22]; even for the same group, various concentration levels are often observed between different countries and WWTPs. The detection of target compounds in sewage sludge raised the public consideration of their possible threats to the environment and human health since biosolids containing TrOCs has been currently applied for agricultural purposes. Considerable efforts have been made to assess the fate of TrOCs during the sludge treatment process in general and anaerobic sludge digestion. A question remains whether their occurrence in sewage sludge would become one of inhibitions or a potential substrate for AD.

Until now, most reported data in the literature have indicated that some surfactants and pharmaceuticals, generally antibiotics, pose an inhibitive effect on AD, while only minor impact caused by other contaminants. In a study investigating the effect of LAS on the anaerobic sludge digestion, a partial and total inhibition of methanogenic activities was observed at high concentrations of LAS homologues [115]. By using a mixed, mesophilic methanogenic culture, Tezel et al. [116] showed that quaternary ammonium-based compounds were more inhibitory to methanogenesis, at and above 25mg/L, than acidogens. Several anaerobic microbes could be sensitive to certain antibiotics at a wide range of concentrations, influencing the overall AD performance. For example, carbamazepine, sulfamethoxazole and diclofenac were studied at mesophilic conditions with a wide range of concentrations by Fountoulakis et al. [117]. The authors observed 50% inhibition on methanogenic activity from 30 mg/L to more than 400 mg/L. A higher range of concentrations of

antibiotics, between 24 mg/L and more than 1,000 mg/L, showed moderate inhibition effects [118].

Little and even conflicting studies on the behaviour of trace organics by AD have been carried out although PPCPs in sewage sludge have been described to be one of the most ubiquitous contaminants [25]. Some PPCPs have been reported to exhibit some resistance to the anaerobic conversion process. In the liquid phase of digested sludge, most available PPCPs were observed to be persistent [119]. The authors stated that diclofenac offered severe inhibition to AD at high concentrations while the two other PPCPs had no effect even at the high concentrations. Nonetheless, removal efficiencies of PPCPs during this digestion have also documented by a variety of comprehensive studies, varying from very high removal to nearly no removal. Excellent PPCPs removal was confirmed for naproxen, sulfamethoxazole, roxithromycin and oestrogens at more than 85%, followed by galaxolide, tonalide and diclofenac at 60%. The value ranged between 20 and 60% with the exception of carbamazepine, which showed no degradation [24, 25]. These eliminations achieved were attributed to sludge adaptation to AD. In another study on the fate of pharmaceuticals, ibuprofen and naproxen removal were once confirmed with considerably percentages more than 80% [21].

Regarding surfactants, it is believed that AD of sludge could degrade some persistent compounds. LAS is one particular example with different removal reported in several studies [25, 120, 121]. More data relating to the mechanism and removal efficiencies of nonylphenolethoxylates during the anaerobic conversion have been reported. In general, nonylphenolethoxylates is biotransformed into shortened chain formations such as nonylphenolpolyethoxylates and nonylphenol [122]. Nonylphenolpolyethoxylate in some studies were metabolized into nonylphenol [27]. Nonylphenol could be degraded during the anaerobic sludge digestion process to some extent [23, 26, 27]. There is little literature assessing the biodegradation kinetics of these compounds with the exception of nonylphenol, whose biodegradation followed the first order kinetic model [26].

The most striking example of contradictory results for the fate during the anaerobic sludge treatment is the group of estrogenic compounds. The rationale likely lies in inherent difficulties of analysing these compounds in sludge matrix and their unclear behaviours. From equivalent loads of estrogens in both inlet and outlet of digesters,

Andersen et al. [123] concluded that estrogens were not degraded under methanogenic conditions. 17-β-estradiol-3-sulphate and estrogen-3-sulphate concentrations were even increased by activities of strictly anaerobic desulphating strains [124]. For estrone, 17-β-estradiol, and estriol, a significant extent of removal efficiencies was presented under thermophilic condition and various sludge retention times [24] or with different types of sludge under mesophilic and thermophilic conditions [23] at laboratory scale. Nevertheless, some authors confirmed the opposite. From equivalent loads of estrogens in both inlet and outlet of digesters, Andersen et al. [123] concluded that estrogens were not degraded under methanogenic conditions. Using mass balance, Muller et al. [125] indicated that their elimination were not effective (ca 40%) at the plant scale. There is little data reporting the biodegradation of 17-α-ethinylestradiol during the sludge AD process [126]. Even the mass flow in the outlet for 17-α-ethinylestradiol was observed to be higher compared to the inlet in one recent study [125].

In sewage sludge, phthalic acid esters were detected in sludge at higher concentrations in comparison to certain compounds mainly originating from industrial applications, such as organotins and brominated flame retardants [22]. The mechanism of anaerobic biodegradation pertaining to phthalates has aroused greater consideration to date. Some literature has presented different removal efficiencies of phthalates during sludge AD. Dimethyl, diethyl and di-n-butyl phthalate esters were degraded within one week [127], nearly completely in a lab-scale experiment [128, 129]. It should be mentioned that the most hardly removed phthalic acid ester is bis(2-ethylhexyl) phthalate, even persistent during the anaerobic biodegradation [127]. By using mass balance in a full-scale WWTP, Marttinen et al. [130] showed that the percentage of bis(2-ethylhexyl) phthalate removal just reached around 32% via AD of sewage sludge. Only when some operational parameters, such as temperature and type of inoculum and HRT are improved, the elimination in some extent of this compound was identified [131, 132].

Most studies on TrOCs removal during the anaerobic sludge digestion process were operated at the full-scale WWTP. This is probably the greater demand of assessing the fate of TrOCs in the discharge flow. Although their eliminations at laboratory scale have been poorly studied, the greater efficiencies have been recognised for many compounds, which were reported to be refractory [23, 24, 128]. Some practical

methods, such as pretreatment processes or a combination of AD with other treatments could be applied during recent years. The co-digestion of sludge with some kinds of readily biodegradable carbon source was also indicated to enhance the anaerobic biodegradation [26, 121], limited research regarding this enhancement has been reported to date though. This may be explained by improving the overall mechanism through accelerating the hydrolysis step. For example, Chang et al. [26] demonstrated this effectiveness on nonylphenol removal, whereas the optimised elimination of polycyclic aromatic hydrocarbons was suggested to reach via the co-metabolism by Barret et al. [112].

2.5 Summary

The AD process, a sequential-stage process with several complex biochemical processes involved in the absence of oxygen, has aroused much interest due to its advantages including the production of renewable energy in the form of CH_4 and lower installation cost compared to its counterpart, the aerobic digestion process. The efficiency and instability of anaerobic sludge degradation greatly depend on several operating conditions (pH and temperature for example) and properties of sewage sludge. Higher performance in terms of CH_4 production has been achieved anaerobically co-digesting sewage sludge with various organic waste materials. Of them, glycerol was a considerable co-substrate due to high in solubility and rich in easily degradable organic components, little research has been in the literature to date though. In respect of removal efficiency by the AD process, more consideration must be given to TrOCs occurring in sludge because of their detrimental effects on ecosystem. Little and even conflicting studies on the elimination of TrOCs under anaerobic condition have been assessed at laboratory scale although these trace contaminants determine the suitability of biosolids for agricultural applications.

3 MATERIALS AND METHODS

This chapter details experimental design, experimental protocols, and analytical methods applied throughout this study. Materials, namely inoculum, substrate and selected TrOCs and their properties were also introduced.

3.1 Materials

3.1.1 Inoculum

Digested sludge from a Sydney Water sewage treatment plant was used as inoculum. This inoculum was withdrawn from a full-scale anaerobic digester operating under mesophilic condition. The digester was continuously fed with only primary sludge, thus, the collected inoculum has already acclimatised with raw primary sludge. The inoculum was stored at 4 °C in the dark until utilisation.

3.1.2 Substrates

Raw primary sludge was also collected from a Sydney Water sewage treatment plant. Pure glycerol (>99%) was obtained from VWR International (Australia). Two kinds of crude glycerol were also obtained from the biodiesel industry (through Sydney Water). They are identified as Biodiesel and BIA, respectively. All substrates were stored at 4 °C in the dark until utilisation to avoid any unexpected biochemical reactions.

3.2 Experimental methods

3.2.1 Biomethane potential test equipment

The BMP test system constructed for this study consisted of fermentation bottles submerged in a water bath and a biogas collection gallery (Figure 5 and Figure 6). The fermentation bottle (Wiltronics Research Pty Ltd) was made of glass and had volume of 1000 mL. Each bottle was equipped with a rubber bung and an S-shape air-lock. The air-lock is filled with water to allow biogas to escape but prevent air from entering the fermentation bottle (Figure 7). The bottle was submerged in the water bath (Model SWB20D, Ratek Instrument Pty Ltd) to maintain the temperature at a constant value. Each bottle was connected to a plastic valve and a gas collector through flexible plastic tube. The biogas collector comprised a 1000 mL plastic

measuring cylinder and a plastic container containing NaOH solution for biogas wash [20, 133, 134] at concentration of 1M in this study. To measure the volume of CH_4 generated from the fermentation bottle, the cylinder was first filled with 1M [134] NaOH solution, and was inverted and then partially submerged into the NaOH container. Biogas from the fermentation bottle was introduced into the submerged part of the cylinder, thus, allowing the NaOH solution to absorb CO_2 and H_2S from the biogas. The remaining CH_4 gas displaced the NaOH solution inside cylinder and the CH_4 gas volume generated each day can be recorded. The water bath could hold up to eight fermentation bottles. Up to eight CH_4 measuring cylinders could also be held by the biogas collection gallery. Thus, the constructed BMP test unit could simultaneously hold eight fermentation bottles. Two identical systems were constructed and used in this study.

3.2.2 Experimental protocols

To minimize oxygen contamination, prior to the BMP experiment, all fermentation bottles were continuously flushed with N_2 for 5 minutes before filling with 750 mL of substrates and inoculum. The headspace in each bottle was 250 mL. The bottle was flushed again with N_2 and immediately sealed with the rubber stopper (Figure 7). After placing into the shaking water bath, which was set at 35.0 ± 0.1 °C, the valve was opened to allow biogas to enter the gas collection gallery. Shaking velocity of the water bath was 70 – 75 strokes per minute. The experiment was terminated when less than 5 mL of CH_4 was produced over a day.

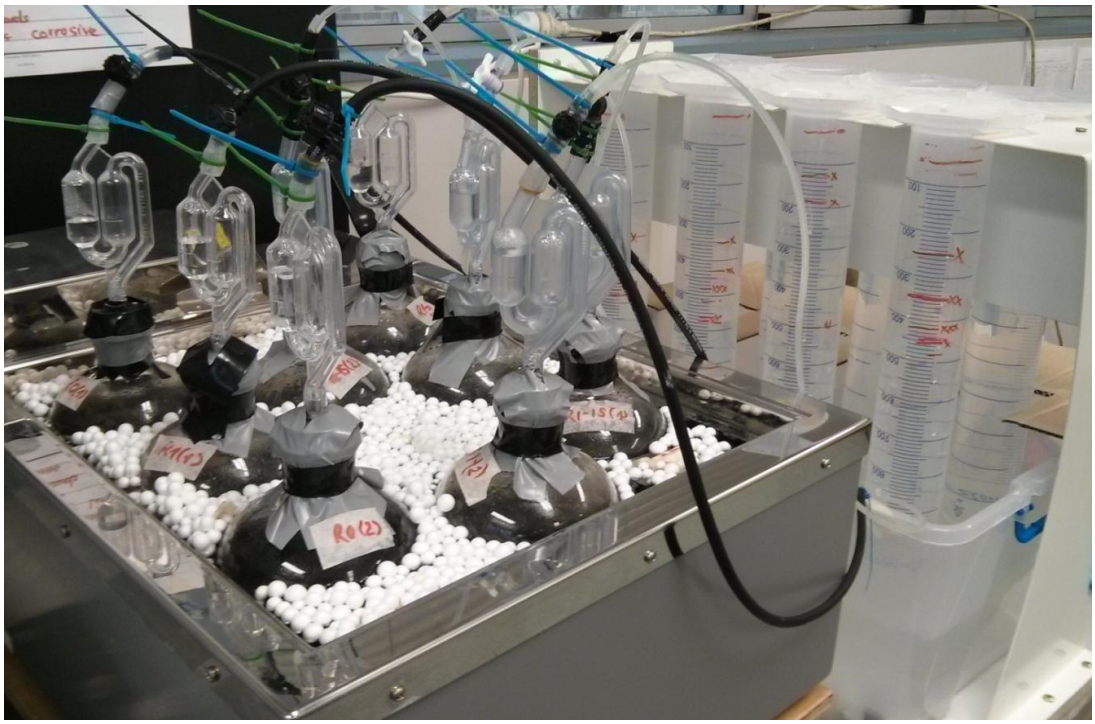


Figure 5. A picture of the BMP test system.

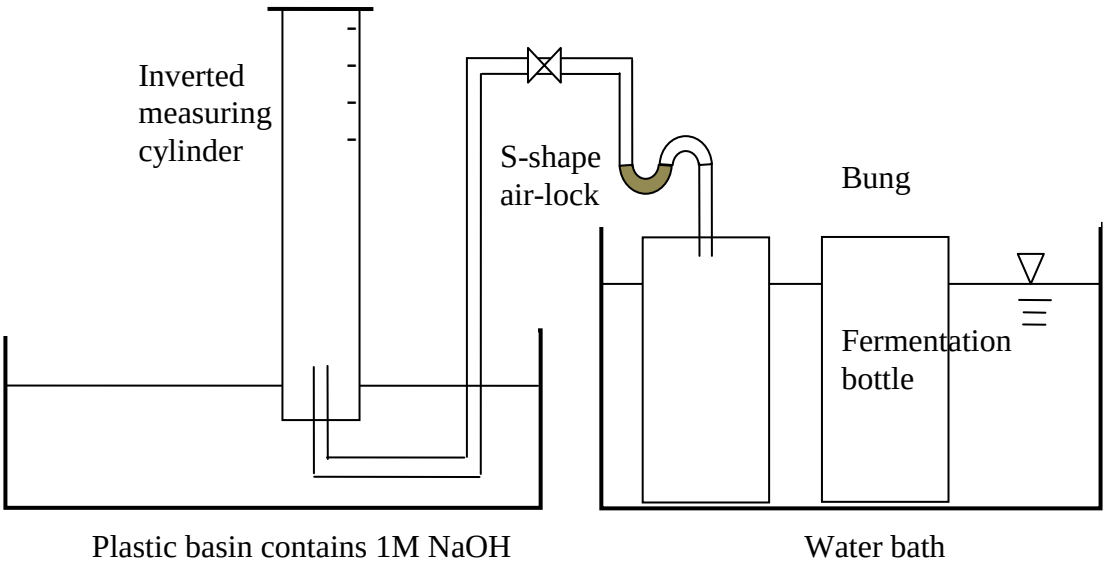


Figure 6. The schematic diagram of BMP test system.

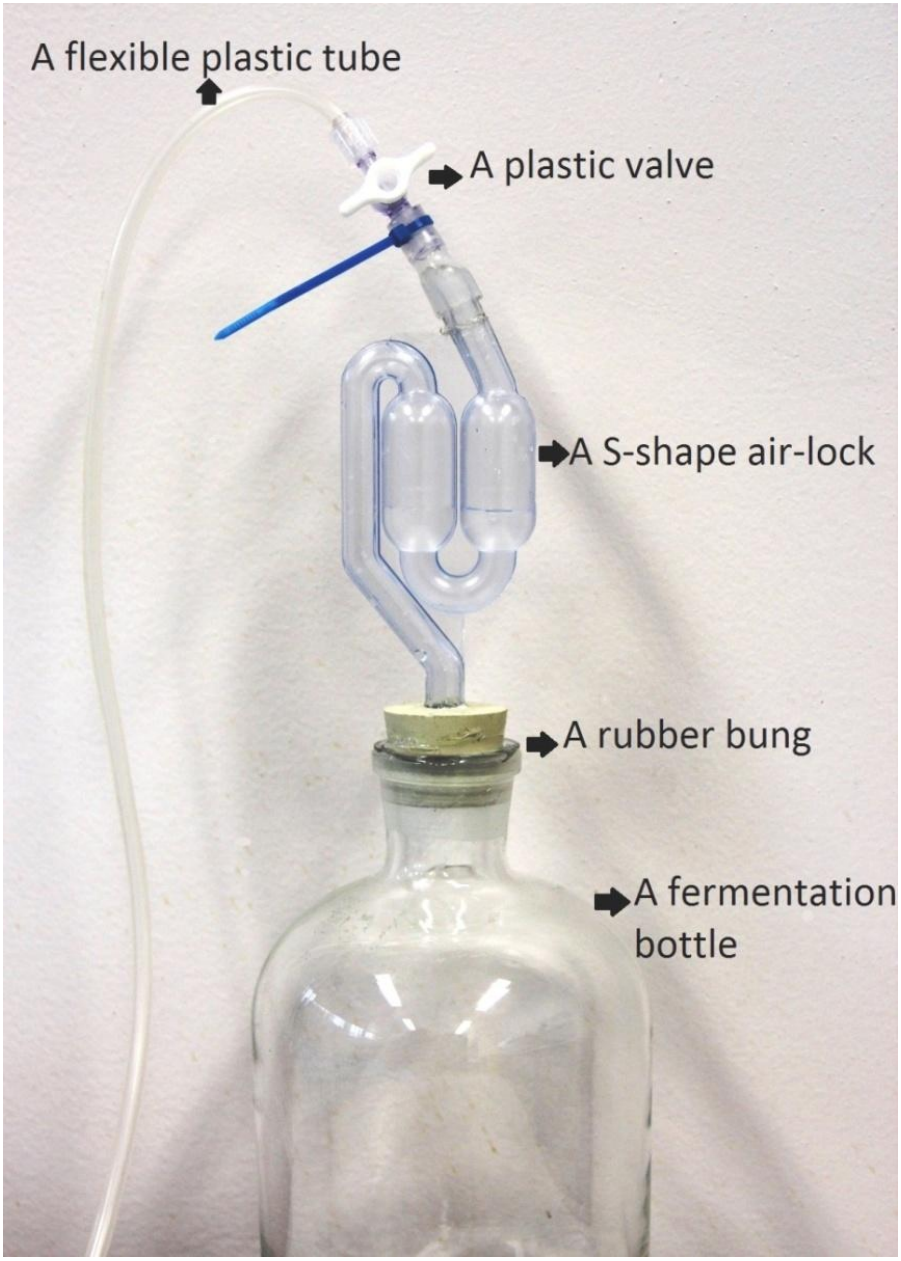


Figure 7. A picture of one BMP bottle unit used in this study.

3.2.3 Experimental description

The anaerobic batch experiments were initialised to perform BMP tests of substrates, including single digestion of each raw primary sludge and glycerol, and their co-fermentation under anaerobic condition. The AD system was then used to evaluate the TrOC removal efficiency of anaerobic sludge treatment. Figure 8 presents the experimental scheme of this investigation. Further details are given as following.

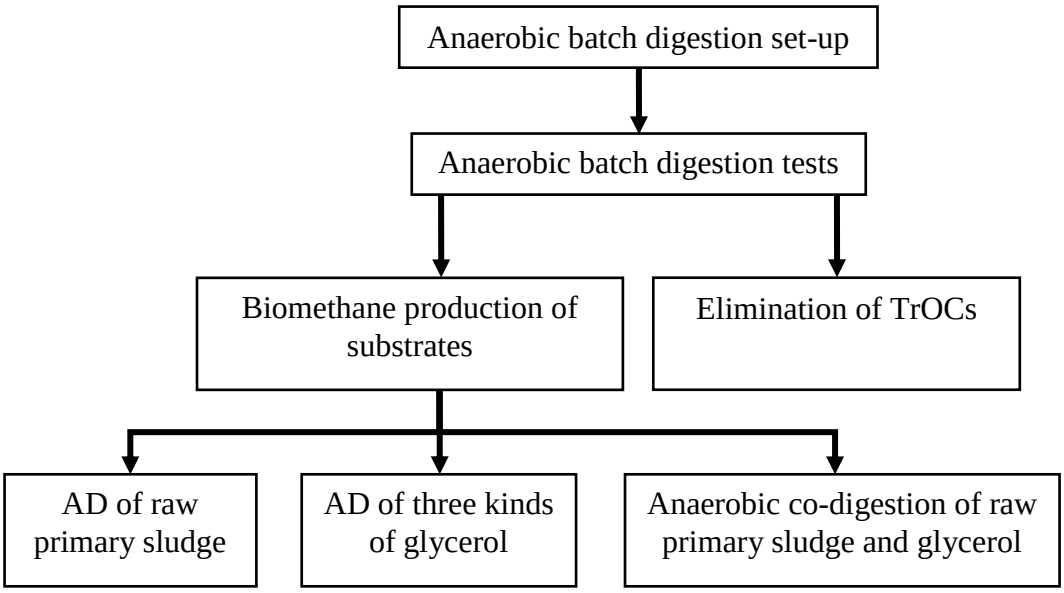


Figure 8. Experimental roadmap.

3.2.3.1 Batch tests on CH_4 potential of sewage sludge and glycerol

Experimental protocol of a BMP assay to evaluate the effect of buffer addition on AD of sewage sludge alone is described in Table 8. The BMP bottles were seeded with a mixture of digested sludge and raw primary sludge (known as I/S ratio) of 1/9 by volume. Because of their low pH and alkalinity, 6.2 and 1228.6 mg/L respectively, sodium bicarbonate ($NaHCO_3$) was added into these bottles to gradually enhance the buffering capacity at the beginning of AD process. $NaHCO_3$ was added at increasing concentrations, 15 and 30 mM. All these batch tests were conducted in duplicate. Data were expressed as mean values.

Table 8. Experimental description of BMP bottles with increasing buffering capacity.

No	Labels of BMP bottles	I/S (by volume)	Added $NaHCO_3$ (mM)
1	R-0	1/9	0
2	R-15	1/9	15
3	R-30	1/9	30

Another bottle with only sludge mixtures was made as a baseline for comparison. A blank bottle with digested sludge was prepared for CH_4 yield from digested sludge only to determine the net CH_4 yield. During the experiment, CH_4 volume was recorded daily until CH_4 production was detected at less than 5 $mLCH_4$/d.

Another set-up was used to assess BMP of raw primary sludge under anaerobic condition. The ratio between inoculum and substrate was increased up to 1/1 by volume. Moreover, digested sludge used in this set-up was already degassed for the best microbial activity, namely exhausted digested sludge. There were two BMP bottles of this mixture but no blank sample as a result. These analyses were conducted in duplicate. Reading of CH_4 production was taken daily.

With the purpose of enhancing the CH_4 yield, co-digestion was taken place with pure glycerol (chemical grade) as a co-substrate for sewage sludge. Batch tests were also conducted to examine how glycerol seeded bottles perform and stabilise. In terms of performance, CH_4 yield and CH_4 production rate were evaluated. Mixture sludge at ratio of I/S (1/9 by volume) was fed to all bottles in this experiment. Once again, buffer supplement by $NaHCO_3$ (15 and 30 mM) was applied. Unlike the previous BMP assays, increasing percentages of pure glycerol (0.5% and 1.0% of raw primary sludge) were added (Table 9). This addition did not change the whole working volume. Other two reference bottles without pure glycerol were taken into account for a baseline of CH_4 generation with an introduction of 15 and 30 mM $NaHCO_3$, while CH_4 yield of digested sludge only was given from the previous batch test. Therefore, three sets of bottles, namely R-15 and R-30 for buffer supplement at 15 and 30 mM without added glycerol, R-15-0.5 and R-30-0.5 for 0.5% glycerol treatments, and R-15-1.0 and R-30-1.0 for 1.0% glycerol treatments, were established.

Table 9. Experimental description of BMP bottles with glycerol addition.

No	Labels of BMP bottles	I/S (by volume)	Glycerol (%of RS)	Added $NaHCO_3$ (mM)
1	R-0	1/9	0	0
2	R-15	1/9	0	15
3	R-30	1/9	0	30
4	R-15-0.5	1/9	0.5	15
5	R-15-1.0	1/9	1.0	15
6	R-30-0.5	1/9	0.5	30
7	R-30-1.0	1/9	1.0	30

One more series of BMP bottles was set up for identifying the actual CH_4 yield of glycerol. In this case, three different kinds of glycerol, i.e. pure, biodiesel and BIA glycerol, were utilized as a carbon source for anaerobic microorganisms or digested sludge. They were determined their viscosity by a rheometer (Section 3.3.5). Their

relative high viscosity (at 35 °C) required a well mixing step when glycerol was introduced into bottles. All BMP bottles were fed with fresh anaerobic inoculum and then glycerol with increasing percentages, i.e. 0.25% and 0.5% of digested sludge by volume. Content and labels of BMP bottles are shown in Table 10. Two control bottles contained only anaerobic inoculum. CH_4 volume was recorded daily until CH_4 was produced at rate less than 5 $mLCH_4$/d.

Table 10. BMP bottles assessing BMP of different types of glycerol.

No	Labels of BMP bottles	DS (mL)	Types of glycerol	Glycerol (% of DS by volume)	Glycerol (mL)
1	P-0.25	750	Pure glycerol	0.25	1.88
2	P-0.5	750	Pure glycerol	0.50	3.75
3	BID-0.25	750	Biodiesel	0.25	1.88
4	BID-0.5	750	Biodiesel	0.50	3.75
5	BIA-0.25	750	BIA	0.25	1.88
6	BIA-0.5	750	BIA	0.50	3.75

Digested sludge (DS) or inoculum

To assess the stabilisation of the AD process, certain parameters such as pH, total alkalinity, total organic acids, COD_t and COD_s were measured at the start and end of each batch test.

3.2.3.2 Anaerobic treatment test of trace organics

The last BMP set-up was for evaluating the fate of trace organics during the anaerobic treatment of sludge. A set of 30 TrOCs was selected as representatives of emerging organic groups, namely pharmaceuticals, steroid hormones, phytoestrogens, UV-filters and pesticides, which are omnipresent in sewage sludge from WWTPs. The selection was also made according to public concerns regarding their applications in the agricultural sector and their diversity in terms of chemical structures and properties (i.e. hydrophobicity and solubility). The hydrophobicity was identified via their log *D* values at pH 8.0, including hydrophilic (log D<3.2) and hydrophobic (log D>3.2) compounds (Table 26 – Appendix). All these TrOCs were purchased at analytical grade. Their combined stock solution (25 μg/mL) was freshly prepared in pure methanol. This solution was kept at –18 °C in the dark and used within one month.

Up to ten BMP bottles used in this arrangement were firstly seeded with anaerobic inoculum. The same volume of the stock solution of 30 TrOCs was spiked into all these bottles to achieve a concentration of 135 μg/L of each trace organic

contaminant. A gentle stirring step was followed in 5 minutes to obtain a homogenous spike. This concentration is equal to 5μg/gTS, as TS of anaerobic inoculum was reported at 27 g/L in this batch test. This investigation was operated for 35 digestion days. At particular time intervals, 0, 2, 6, 9, 14, 21, 28, and 35 days, each bottle was withdrawn for solid phase extraction (SPE) within 24 hours, and subsequently TrOC analysis. In this series, two non-spiked bottles, containing only inoculum, were integrated as blank samples. Stability of this anaerobic mineralization of TrOCs were governed in terms of parameters, including CH_4, TS, VS, COD, pH, alkalinity, and ammonia nitrogen. All analyses were performed in duplicate.

3.3 Analytical methods

3.3.1 Basic parameters

pH was measured by using a Metrohm Advanced pH/Ion Meter at the room temperature, around 25 °C. TS, VS, and alkalinity were measured according to the Standard Methods [135].

For measuring TS and VS concentrations, sludge sample (20 mL) was transferred into pre-weighted crucibles. The crucibles were then placed in a water bath for 1 hour at 100 °C for dewatering before being placed in an oven for 24 hours at 105 °C. The crucible was allowed to cool down to room temperature; and the total weight of the crucibles and dried sample was measured. Accordingly, TS was determined as the dried sludge retained in the crucibles. The crucible was then placed into an oven (1400 furnace, Barnstead Thermolyne, Australia) preheated at 550 °C for 15 minutes. The crucible was allowed to cool down to room temperature and weighted again to determine VS concentration.

Supernatant collected from 50 mL sample after centrifugation was used for alkalinity analysis using the titration method [135]. The solution H_2SO_4 of0.1N was used as the titrating chemical and was calibrated using 0.05N Na_2CO_3. Titration was performed to a pH end-point of 4.5. Alkalinity was calculated according to the following equation:

$$Alkalinity\ (mgCaCO_3/L) = \frac{A \times N \times 50{,}000}{mL\ sample} \quad (1)$$

where: A = mL of standard 0.1 N H_2SO_4 used, and

N = normality of standard 0.1N H_2SO_4

Total organic acids were measured by the method 5560C with distillation step followed by titration step with standard 0.1N NaOH to the end-point pH of 8.3 [135]. Result, expressed by mg acetic acids/L, was from the following equation:

$$Volatile\ acids\ (mg\ acetic\ acid/L) = \frac{mL\ NaOH \times N \times 60{,}000}{mL\ sample \times f} \quad (2)$$

where: N = normality of standard 0.1N NaOH, and

f = recovery factor for a given distillation apparatus

3.3.2 Chemical oxygen demand

The determination of COD_s was performed by centrifuging a volume of sample (20 mL) and filtering through a Milipore HA membrane 0.45 μm pore size [136]. Approximately 10 mL of sample was also used to measure COD_t. After appropriate sample preparation, each sample and one blank (deionized water) were taken for 0.2 mL and introduced into High Range Plus COD Digestion Reagent vials (200 – 15000 mg/L), and heated at 150 °C in a Hach DBR200 COD Reactor for 2 hours. After this digestion step, vials were cooled down to room temperature to proceed to colorimetric determination by using a Hach DR/2000 spectrophotometer with method 435 COD HR (Figure 9). The result was expressed as mg COD/L.

a) b)

Figure 9. Hach equipment for COD determination: a) DBR200 COD Reactor and b)DR/2000 Spectrophotometer.

3.3.3 Methane yield calculation

During the experiments, the volume of CH_4 generated can be calculated from measurements of the change in liquid height in the column and container.

One blank bottle containing only DS with the equivalent volume of sample bottles was included to account for the CH_4 yield generated from inoculum alone. The net CH_4 volume was corrected by subtracting the CH_4 volume in the blank from the CH_4 volume in the sample at the same time of sampling:

$$Net\ CH_4\ production\ (mL) = CH_4\ of\ sample - CH_4\ of\ blank \quad (3)$$

BMP, which is based on the chemical nature of substrates, was determined by normalising the generated CH_4 volume against the initial VS or COD weight basis:

$$Cumulative\ CH_4\ yield = \frac{Net\ cumulative\ CH_4\ (mL)}{Mass\ of\ COD\ or\ VS\ added\ (g)} \quad (4)$$

Specific methanogenic activity (SMA) is calculated as the following [137]:

$$SMA = \frac{R}{350 \times V \times VS_{inoculum}} (gCOD / gVS.d) \quad (5)$$

where R is daily CH_4 production ($mLCH_4/d$), V and $VS_{inoculum}$ are volume and VS concentration of inoculum (known as digested sludge), and 350 is a conversion factor according to Mc Carty [138] that every one gram COD is theoretically converted into 350 mL CH_4 in one anaerobic digester.

CH_4 yield was modelled using the first order degradation equation according to [139],

$$G(t) = G_o \times \left(1 - e^{-kt}\right) \quad (6)$$

where G(t) is the cumulative CH_4 yield at time t ($mLCH_4/gVS_{added}$ or $mLCH_4/gCOD_{added}$), G_o is the ultimate CH_4 yield ($mLCH_4/gVS_{added}$ or $mLCH_4/gCOD_{added}$), which is defined as the final volume of CH_4 beyond which no more CH_4 is released, k is the first order rate constant, and t is the digestion time at which the corresponding cumulative CH_4 production is recorded.

The modified Gompertz equation was established by assuming that the CH_4 production in batch condition is a function of growth rate of methanogenic bacteria over time:

$$G(t) = G_o \times e^{-e^{\left(\frac{R_{max} \times e}{G_o} \times (\lambda - t) + 1\right)}} \quad (7)$$

where G(t) is the cumulative CH_4 yield at time t ($mLCH_4/gVS_{added}$ or $mLCH_4/gCOD_{added}$), G_o is the ultimate CH_4 yield ($mLCH_4/gVS_{added}$ or $mLCH_4/gCOD_{added}$), R_{max} is the maximum CH_4 production rate (mL/d), λ is the

duration of lag phase (d), t is the digestion time at which the corresponding cumulative CH_4 production is recorded.

The fitness of these above equation in regulating the CH_4 production was assessed using the nonlinear curve-fitting tool of Matlab. At the same time, all parameters, the standard error and the coefficient of determination or correlation coefficient (R^2) were also calculated.

Average values of CH_4 yields in all experiments were statistically compared by using one-way ANOVA (Analysis of Variance) in Excel. The calculated F value and tabulated F value were compared to judge whether difference between two values is significant or not. If the F value is greater than F critical or calculated P-value is greater than level of significance (α), there is at least a significant difference between two means. In this case, the least significant difference (LSD) of any of them was calculated at $\alpha = 0.05$ and $\alpha = 0.01$ as following equation:

$$LSD_\alpha = t_\alpha \times \sqrt{\frac{2 \times s^2}{r}} \quad (8)$$

where t_α is tabulated value identified from the degree of freedom for error (d*f*) and α, s^2 is the mean square for error (MS), and r is the number of replications on which the means were computed. MS and d*f* were calculated by using one-way ANOVA tool in Excel.

3.3.4 Trace organic analysis

Concentrations of TrOCs in the liquid phase were measured by using the method reported by Hai et al. [140]. The schematic procedure of these methods is described in Figure 10.

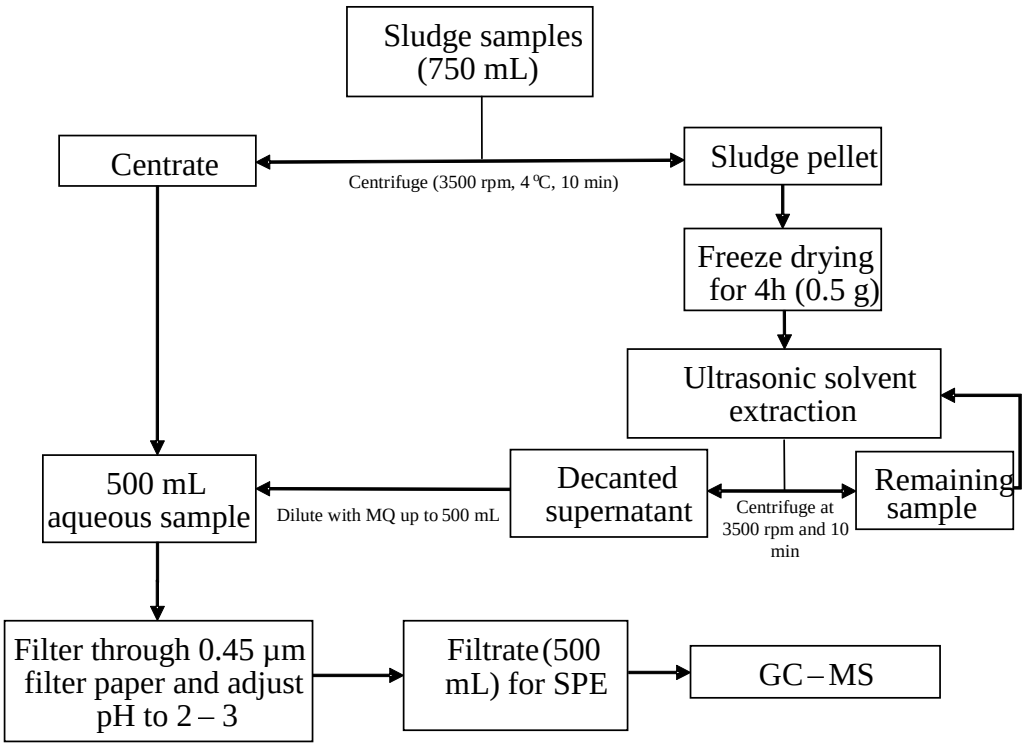


Figure 10. A schematic of sample preparation in solid and liquid phases GC-MS analysis.

Samples were analysed at the particular times during the experiment. At every single analysis, standard TrOC solution samples were included for the accurate measurement. At first, sludge samples were centrifuged to separate supernatant and sludge pellet. In the supernatant, these compounds were initially extracted from 500 mL sample volume using Oasis HLB cartridges (Waters, Milford, MA, USA). They were then eluted from the cartridges and the eluents were evaporated to dryness. After that, the dry residues in the vials were derivatised, cooled to room temperature, and subjected to gas chromatography–mass spectrometry (GC–MS) analysis using a Shimadzu GC–MS (QP5000) system. TrOC concentrations in solid phase were determined according to the method previously described by Wijekoon et al. [141]. An extraction step, including sludge pellet freeze-drying and ultrasonic solvent extraction was used. Sample was then analysed using the same method for the liquid phase. All analysis was conducted in duplicate.

3.3.5 Viscosity of glycerol

Viscosities of pure, biodiesel, and BIA glycerol as a function of temperature were measured by using a Physcia MCR 301 Anton Paar Rheometer (Figure 11). The

rheometer was controlled by the RHEOPLUS/32 V3.40 software. The sample volume was 1 mL.

Figure 11. A photograph of the Anton Paar Physica MCR 301 Rheometer.

4 BIOMETHANE POTENTIALOF SEWAGE SLUDGE AND GLYCEROL

This chapter first describes the key characteristics of sewage sludge and glycerol. Thereafter, batch-mode BMP tests were performed in order to identify their biodegradability with respect to CH_4 yield. Effects of buffer supplement and inoculum over substrate (I/S) ratio on CH_4 yield and process stability were also evaluated for sewage sludge, respectively. Meanwhile, the biodegradability of three types of glycerol, namely pure, biodiesel and BIA, were assessed and compared.

4.1 Sewage sludge

4.1.1 Characteristics of sewage sludge

Key properties of raw primary sludge, digested sludge (which was used as inoculum), and exhausted digested sludge are summarised in Table 11. The TS and VS over TS ratio of raw primary sludge were 25.3 g/L (or 2.5%) and 90.4%, respectively. The TS content of raw primary sludge reported here is consistent with the literature (for example TS content in the range of 2.0 – 8.0%) as can be seen in Table 1 of Chapter 2. The raw primary sludge used in this study has a high VS fraction, which is evidence in a VS/TS ratio that is slightly higher than the range of 60 – 80% typically reported in the literature (Table 1). On the other hand, a lower VS/TS ratio was found in the digested sludge (ca 60%) compared to raw primary sludge. This is typical for inoculum. As expected, the digested sludge also had a much lower soluble organic fraction than the raw primary sludge (i.e. 0.8 $gCOD_s/L$ compared to 2.7 $gCOD_s/L$).

Values of pH of the raw and digested sludge differ markedly from each other. Due to the primary treatment process used in the sewage treatment plant, raw primary sludge was relatively acidic (pH = 5.8) and had a low alkalinity (953.8 $mgCaCO_3/L$). On the other hand, a high pH and much higher buffering capacity (4185.7 $mgCaCO_3/L$) were observed in digested sludge. This high alkalinity value reflects the stability of the AD process. Because of the production of VFAs, which accelerates the acidification stage, inadequate buffering capacity system may lead to system failure. In order to sustain the stability of anaerobic conversion process, the ratio of VFAs and total alkalinity should be maintained at below 0.4 [142]. The VFA/total alkalinity ratio of the digested sludge reported here was 0.17 (Table 11), indicating that the inoculum were from a healthy anaerobic digester.

Ammonia concentration is another parameter to assess the biological performance of the AD process. As can be seen from Table 11, raw primary sludge used in this study had low ammonia content (i.e. 543 mg/L). Regarding the effect of ammonia nitrogen levels on the anaerobic system, Procházka et al. [143] reported that inherent buffering capacity inside the anaerobic digester is a function of organic acids, ammonia and bicarbonate content (Section 2.2.3). While these authors confirmed the inhibitive effect of high ammonia level of 4 g/L, to microorganism, they also reported the low CH_4 production resulted from 0.5 g/L ammonia nitrogen. These conclusions were dependent on inoculum origin and types of substrates [58]. In the present study, the inoculum has a high ammonia content (610 mg/L) which was possibly produced during the methanogenesis step from nitrogen-containing substrate [42].

After collection, digested sludge further incubated for 24 days to obtain exhausted digested sludge (Section 2.2.3). VS/TS ratio of exhausted digested sludge was lower than that of initial digested sludge. Moreover, its buffer capacity relatively increased up to approximately 6000 mg$CaCO_3$/L possibly due to the production of ammonia (i.e. 890 mg/L). This incubation process was considered to be favourably operating without any inhibition since the VFAs/alkalinity ratio was 0.18. Differences in terms of pH and COD between initial and exhausted digested sludges were negligible.

Table 11. Characteristics of raw, digested, and exhausted digested sludge.

Characteristics	Raw primary sludge	Digested sludge	Exhausted digested sludge
TS (g/L)	25.3	21.3 – 27.0	22.9
VS (g/L)	22.9	13.6 – 17.5	13.4
VS/TS (%)	90.4	63.8 – 64.8	58.5
pH	5.8	7.6	7.6
Alkalinity (mg$CaCO_3$/L)	923.8	4185.7	5947.6
COD_t (g/L)	22.7	22.4	21.7
COD_s (g/L)	2.7	0.8	0.8
COD_s/COD_t (%)	11.9	3.6	3.7
NH_4^+-N (mg/L)	543	610	890
Total organic acids (mg/L)	nd	703.8	1073.1

nd = no data

4.1.2 Effects of buffer supplement on the anaerobic treatment of sewage sludge

Batch tests were conducted to assess impacts of buffer on biodegradation of RS under anaerobic condition (Section 3.2.3). Table 12 lists the average values of key parameters of raw primary sludge and inoculum mixtures before and after the experimental period.

An increase in pH and buffer capacity occurred along with increasing concentrations of $NaHCO_3$ or bicarbonate added (Table 12). The addition of $NaHCO_3$ had no impact on other parameters, including TS, VS, and COD. A slight increase of pH from 6.2 to 6.6 and 6.9 was a result when supplying 15 and 30 mM $NaHCO_3$ into the initial sludge mixture, respectively. Correspondingly, the buffer capacities increased from 1228 to 1952 and 2929 mg$CaCO_3$/L. As previously discussed in Section 2.2.3, the optimal pH range of anaerobic process are from 6 to 8. Meanwhile, alkalinity values obtained here are in line with those (ca 1000 – 3000 mg$CaCO_3$/L) in the literature [144]. Therefore, the introduction of $NaHCO_3$ facilitated the bacterial activity at the start-up of digestion.

Table 12. Characteristics of sludge samples.

	Characteristics	Sludge mixtures		
		R-0	**R-15**	**R-30**
Content	I/S (by volume)	1/9	1/9	1/9
	Added $NaHCO_3$ (mM)	0	15	30
Initial values	TS (g/L)	24.1	27.8	31.5
	VS (g/L)	21.3	23.1	25.3
	pH	6.2	6.6	6.9
	Alkalinity (mg$CaCO_3$/L)	1229	1952	2929
	COD_t (g/L)	31.4	31.7	29.7
	COD_s (g/L)	2.0	2.2	2.2
Final values	TS (g/L)	17.7	17.0	18.3
	VS (g/L)	14.0	12.6	12.2
	pH	4.4	4.5	4.7
	Alkalinity (mg$CaCO_3$/L)	605	930	1442
	COD_t (g/L)	33.5	31.9	30.6
	COD_s (g/L)	10.8	12.1	12.6
	VS removal (%)	34.1	45.7	51.5
	Net COD_s (g/L)	8.8	9.9	10.4
	Net total alkalinity (mg$CaCO_3$/L)	624	1022	1487

R-0, R-15, and R-30 refer to I/S mixture containing 0, 15, and 30 mM $NaHCO_3$, respectively

The cumulative CH_4 generation per unit mass of VS or COD added as a function of time are presented in Figure 12 and Figure 13, respectively. The obtained data can be described by both the first order and modified Gompertz models (Figure 14). CH_4 production occurred immediately at the beginning of the incubation process, due to the acclimatisation of inoculum to raw primary sludge (Section3.2.3.1). The cumulative CH_4 production increased until day 6 to 8 and no further CH_4 production could be observed. The short duration of batch test in the present study is also consistent with that reported by Lim et al. [145]. They concluded that all BMP bottles ceased working after only 6 to 8 days of biogas production, resulting from either due to adverse conditions developed or the substrates had been depleted [145]. Because of low VS removal (34.1 – 51.5%) observed after incubation, the effect of unfavourable conditions, including acidic pH (ca 4) and low buffer capacities (605 – 1440 mg$CaCO_3$/L) on methanogenesis was one of the reasons.

The peaks of CH_4 production rates were at the first day of digestion for all BMP bottles, calculated as 151.7, 296.8, and 291.8 mLCH_4/gVS_{added}.d respectively for R-0, R-15 and R-30 (Figure 12). This confirms the good adaptation of inoculum to substrates. The lag times derived from the modified Gompertz model showed the consistency with the practical observation, varying from zero for R-30 to 0.01 and 0.04 days correspondingly for R-0 and R-15 (Table 13). The data indicated that the high bicarbonate concentration can shorten the lag phase of methanogenesis, which was also confirmed by Lin et al. [146] as neutral pH in digester was reached. Hao et al. [147] investigated the extension of lag phase at pH as low as 5.5. The addition of $NaHCO_3$, a source of easily degradable substrate, could cause this reduction of lag phase. Lin et al. [146], however, reported that CH_4 yield slow down with increasing bicarbonate levels, which may be due to used higher-concentrated bicarbonates (150 and 200 mM). CH_4 yield from buffered reactors was significantly higher than from the reactors without bicarbonate addition, which may originate from higher buffer capacity (15 and 30 mM). In turn, the difference of CH_4 yields between bottles with 15 and 30 mM of bicarbonate, was found insignificant (*P*-value = 0.312 > 0.05, $LSD_{\alpha=0.05}$ test[1]). Comparatively, the ultimate CH_4 production did not rely on the level of bicarbonate although there was a slight increase in COD, demonstrating that low

[1]Least of significant difference at $\alpha = 0.05$, was computed using the single-factor ANOVA tool in Excel 2010

$NaHCO_3$ only has a buffering effect. The amount of necessary buffer should be higher, consequently. In fact, $NaHCO_3$ concentrations (15 and 30 mM) in this study were still lower than that reported in other studies [146, 148]. Lin et al [146] applied a range from 0 – 200 mM $NaHCO_3$ with an interval of 50 mM, while 150 mM $NaHCO_3$ and 150 mM K_2CO_3 were used by Vavilin et al. [148] from which well-buffered conditions were maintained. Similarly, in terms of VS basis, R-15 and R-30 bottles had higher performance in methanogenesis with 29.1 and 26.6 $mLCH_4/gVS_{added}$, respectively, than R-0 bottles with 21.8 $mLCH_4/gVS_{added}$ (Figure 13).

Compared to data reported in certain previous studies (Table 6), these values are relatively low, which could be attributed to the low concentration of inoculum. A I/S ratio (or the ratio of digested sludge and raw primary sludge by volume) of 1/9 was applied in this study. These results are only comparable with those of a study assessing BMP of thickened sludge from a municipal WWTP by Lim et al. [145]. Using a I/S ratio of 1/8 (by volume), the authors stated that the AD of thickened sludge had the ultimate CH_4 yield of 21.93 $mLCH_4/gVS_{added}$. They suggested that methanogenesis was significantly inhibited at low I/S ratio due to high VFA concentration and an acidic pH.

Figure 14 shows that both first order kinetic and modified Gompertz models can describe the CH_4 yield very well. The higher coefficient R^2 (0.990 – 0.996) from the first order kinetic model in comparison to that (0.970 – 0.991) from the modified Gompertz model indicated that the best fitting model to the substrates used in this batch test is the first order kinetic model (Table 13). This model was known to successfully describe the rate of hydrolysis in the anaerobic degradation [149]. Based on a direct relationship between the CH_4 production and substrate consumption in anaerobic digesters (Section 2.2.1), this model was also useful to evaluate the kinetic of this batch test.

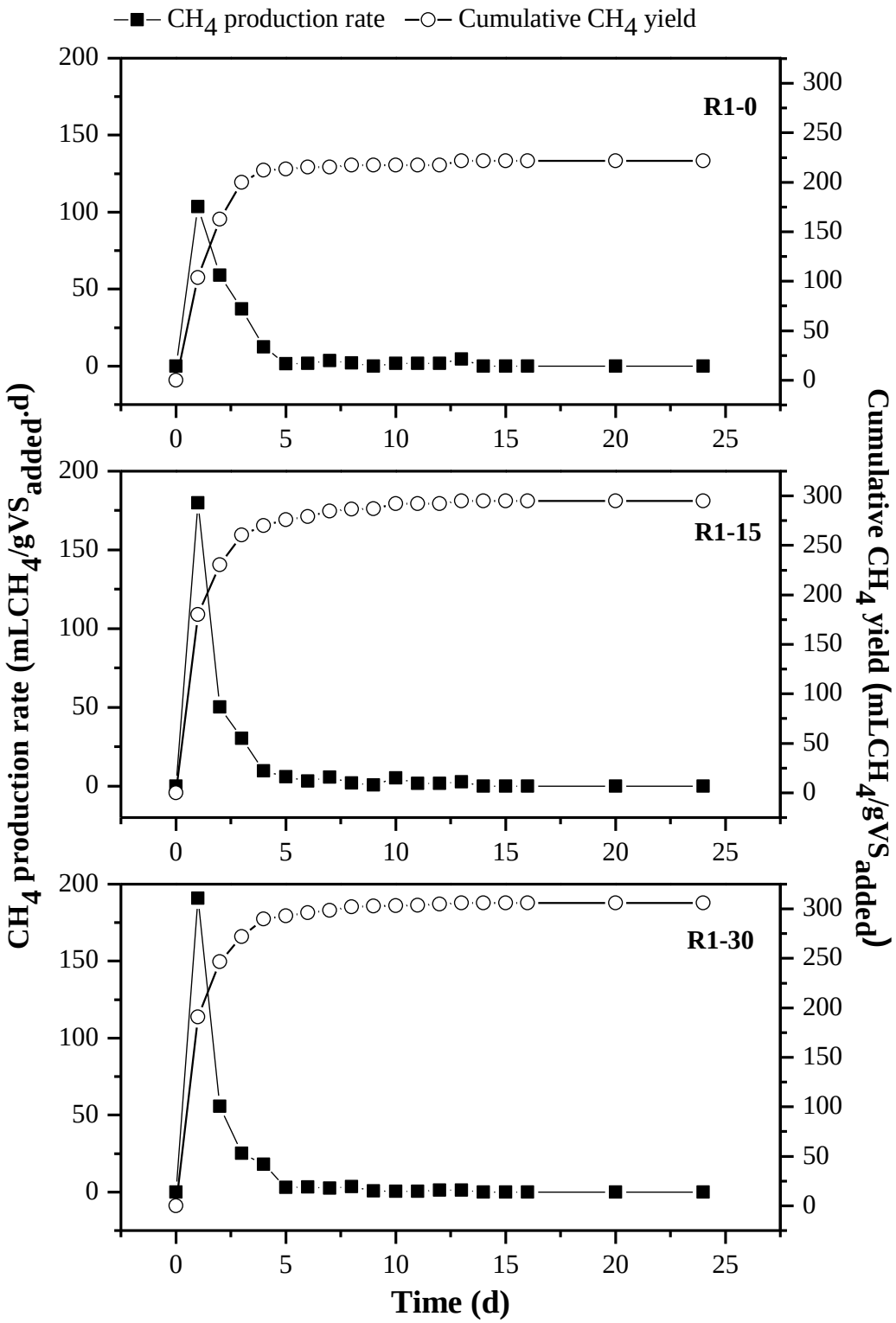


Figure 12. Cumulative CH_4 yield and CH_4 production rate for buffered and non-buffered reactors with increasing buffer concentrations, 0, 15, and 30 mM $NaHCO_3$, respectively labelled as R-0, R-15, and R-30.

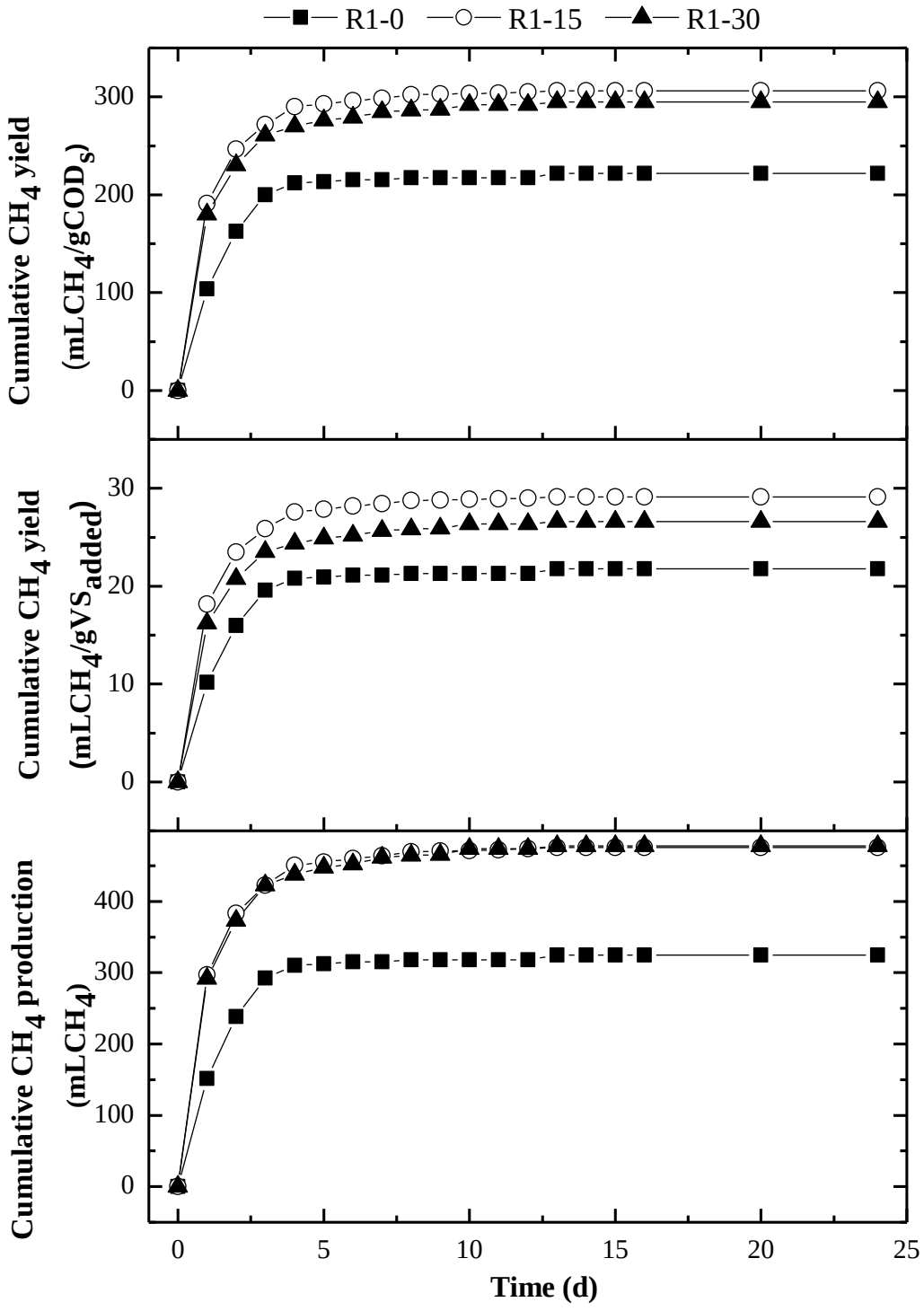


Figure 13. Temporal variations of cumulative CH_4 production of the sample mixture R1 (1/9 I/S) supplemented with increasing buffer concentrations, 0, 15, and 30 mM $NaHCO_3$, respectively labelled as R-0, R-15, and R-30.

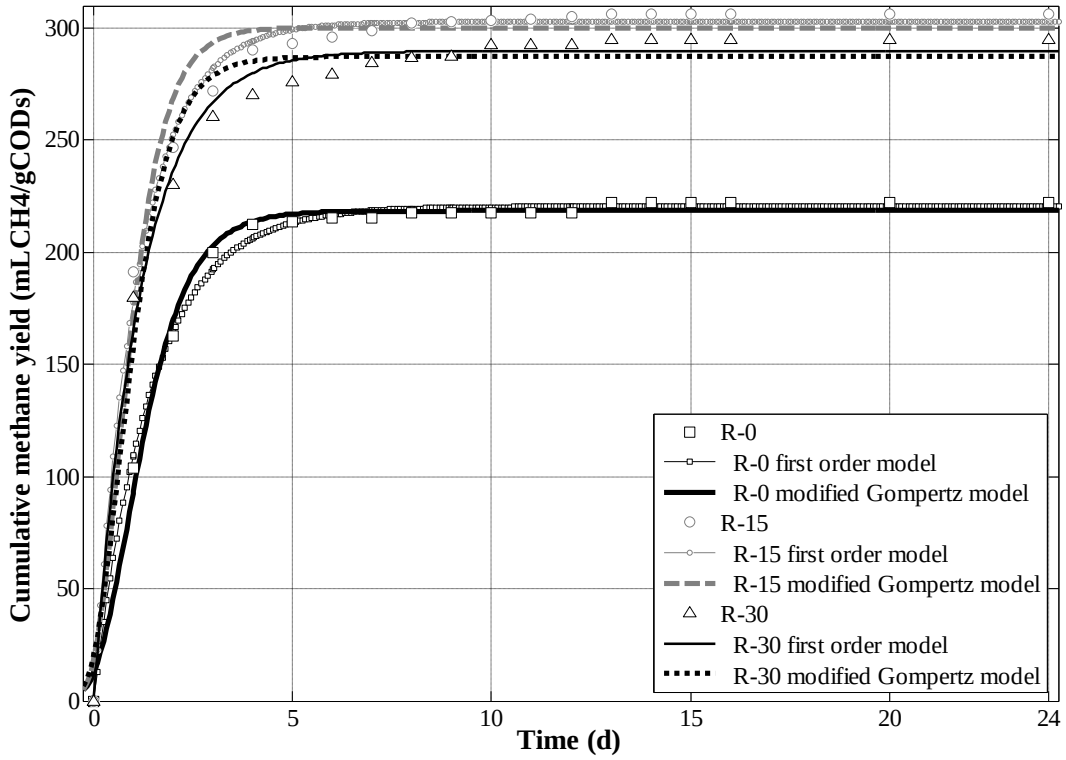


Figure 14. Plots of cumulative CH_4 yields on COD basis and regression fitting curves of the first order and modified Gompertz models.

The greatest VS removal, 51.5%, was achieved in R-30 followed by 45.7% in R-0 and 34.1% in R-15. This low percentage of VS reduction suggests that an incomplete digestion. Meanwhile, all BMP bottles resulted in a high acidic pH (ca 4.5) and low alkalinity. A digester imbalance occurred due to low pH, which inhibited biomass activity. This could be referring to the sharp accumulation of VFAs in the start-up of the digestion course in combination with the insufficient buffer capacity [143, 145, 146]. This observation is in line with that in a study of Procházka et al. [143] who demonstrated that incomplete substrate destruction with reference to VS removal of 53.6% was observed as a consequence of poor biomass activity, acidic pH, and high VFA concentration although pH was neutralised by Na_2CO_3. The authors also indicated even lower removal of substrate in other reactors without buffering agent, only from 13.4% to 29.5% VS removal.

Stable performance of anaerobic treatment could be seen with regards of soluble matter. In particular, initial and final concentrations of COD_s and COD_t were in the proportion with increasing amount of supplemented $NaHCO_3$ (Table 12). Whilst no significant change was observed in COD_t concentrations, the values of net COD_s

(calculated as subtracting the final to initial COD_s concentrations) showed an increasing trend at the end of this batch tests, 8.8, 9.9, and 10.4 mg COD/L respectively for bottles R-0, R-15, and R-30. Since COD_s represents the extent of solubilisation [84], this tendency could be mainly attributed to the VFA accumulation since a decrease in total alkalinity (defined as the difference between final and initial alkalinity) increased proportionally with an increase of COD_sconcentrations (Table 12). This reflects insufficient contributions of acid consumers compared to acid formers, suggesting that there were a complete conversion in the hydrolysis/acidification step and a stress situation in the methanogenic stage. The same conclusion was also stated by Raposo et al. [142, 144].

Table 13. Kinetic analysis of CH_4 production in the batch test of buffer enhancement.

Models and results (at 95% confidential interval)	**R-0**	**R-15**	**R-30**
Experimental cumulative CH_4 yield ($mLCH_4/gCOD_s$)	221.8	306.0	294.8
First order kinetic model			
k (1/d)	0.688	0.896	0.848
R^2	0.996	0.995	0.990
Predicted cumulative CH_4 yield ($mLCH_4/gCOD_s$)	220.2 ± 2.0	302.5 ± 2.9	289.6 ± 3.8
Modified Gompertz model			
Lag phase - λ (d)	0.04	0.01	0.00
R^2	0.991	0.976	0.970
Maximum CH_4 production rate - R_m ($mLCH_4/gCOD_s.d$)	97.8 ± 14.0	177.5 ± 45.0	160.8 ± 23.3
Predicted cumulative CH_4 yield ($mLCH_4/gCOD_s$)	218.3 ± 2.8	300 ± 6.1	286.9 ± 6.4

In terms of the CH_4 production and VS degradation, the $NaHCO_3$ supplement conclusively resulted in a higher performance. Nevertheless, the level of buffering capacity applied here was still not adequate as can be seen in the short CH_4 production duration (Figure 12) and low VS removal efficiency (Table 12).

4.1.3 Methane production of sewage sludge

Initial and final values of parameters of mixture of raw primary sludge and digested sludge of 1/1 ratio by volume are summarised in Table 14. Exhausted digested

sludge used in this batch series was regarded as a healthy anaerobic consortium rather than a source of substrate. That means CH_4 yield was only derived from raw primary sludge.

Table 14. Characteristics of raw primary sludge and sludge mixture.

	Characteristics	Raw primary sludge	Sludge mixture
Initial values	TS (g/L)	38.9	32.4
	VS (g/L)	34.4	25.2
	VS/TS (%)	88.6	77.9
	pH	5.8	8.1
	Alkalinity ($mgCaCO_3$/L)	923.8	3777.0
	COD_t (g/L)	48.9	35.3
	COD_s (g/L)	11.0	5.9
	COD_s/COD_t (%)	22.5	16.7
Final values	TS (g/L)	nd	16.8
	VS (g/L)	nd	10.7
	VS/TS (%)	nd	63.5
	pH	nd	7.9
	Alkalinity ($mgCaCO_3$/L)	nd	5329
	COD_t (g/L)	nd	12.5
	COD_s (g/L)	nd	1.9
	COD_s/COD_t (%)	nd	15.2
Removal efficiencies (%)	TS	nd	48.2
	VS	nd	57.7
	COD_t	nd	64.8
	COD_s	nd	68.8

nd = no data

Slightly lower solid and organic matter content were observed in the raw primary and digested sludge mixture compared to those in raw primary sludge. The sludge mixture thus showed lower ratios of VS/TS and COD_s/COD_t (i.e. 88.6% and 22.5%) than those in raw primary sludge (i.e. 77.9% and 16.7%). In spite of this reduction, the usage of 1/1 (v/v) ratio could offer sufficient bacterial activity from inoculum to well degraded substrate from raw primary sludge. Moreover, the I/S ratio applied in this batch test lead to an enhancement in buffer capacity in the sludge mixture. In particular, pH values and alkalinity values in the sludge mixture were observed at 8.1 and 3777 $mgCaCO_3$/L while those values in raw primary sludge were 5.8 and 923.8 $mgCaCO_3$/L, respectively. It was then expected the stable performance of anaerobic sludge conversion. Data of digestate characteristics also confirmed this process stability. Neutral pH (7.9) and high alkalinity value (5329 $mgCaCO_3$/L) remained in

the expected range for a healthy anaerobic activity. Destruction of both solid and organic matter was achieved, with the removal efficiencies of TS, VS, COD_t, and COD_s were 48.2%, 57.7%, 64.8%, and 68.8%, respectively.

The cumulative CH_4 yield of raw primary sludge was presented inFigure 15. A gradual generation of CH_4 lasted 115 days in this batch test, indicating the high complexity of raw primary sludge. The similar incubation time was applied by [84] whose quantified the ultimate biodegradability of using the batch-mode test. The observed pattern of cumulative CH_4 production was another indicator for this complexity. Two separate periods in CH_4 production might correspond to more easily degradable and slowly degradable materials (Figure 15). This was probably explained by the different biodegradability among organic fractions in raw primary sludge. This observation revealed the anaerobic inoculum was able to recover after the suspended time and utilise all organic fractions available in raw primary sludge subsequently.

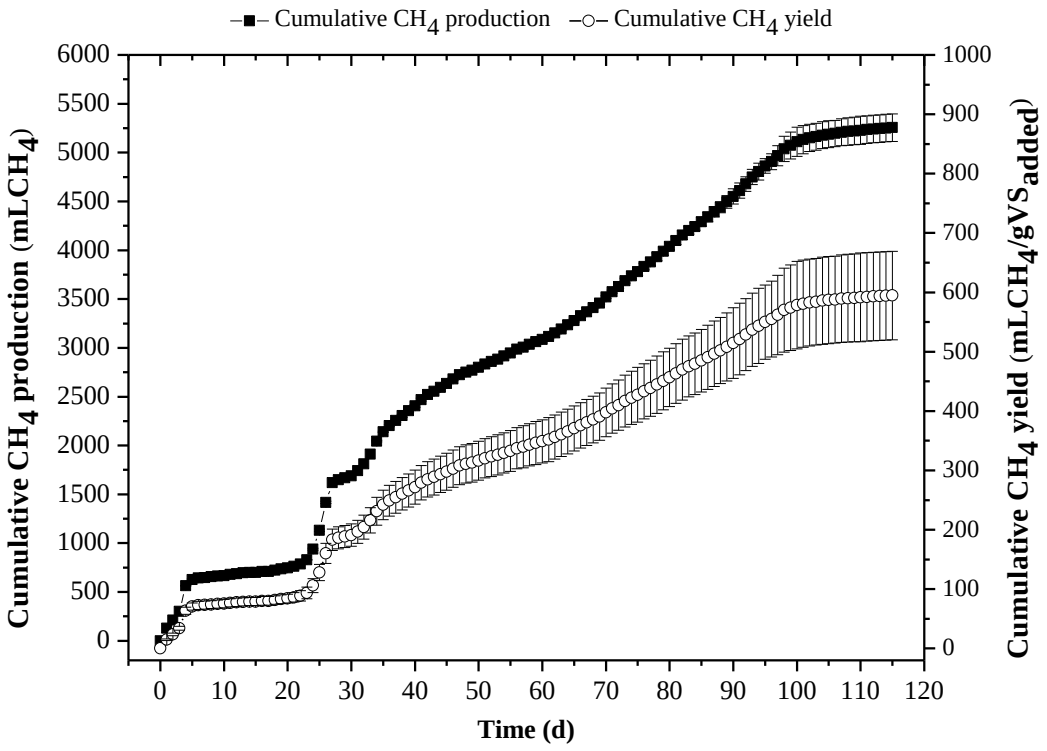


Figure 15. Profile of methanogenesis of raw primary sludge in terms of production and yield over experimental time; error bars refer to the standard deviation (n = 2).

Figure 16 shows the fitted curves of two kinetic models, namely the first order and modified Gompertz models to evaluate experimental CH_4 production data and

predict cumulative CH_4 yields. Comparatively, the regression coefficients from the first order equation could not be computed, demonstrating its failure in reflecting the methanogenesis of such complex substrate as raw primary sludge. Meanwhile, by using the modified Gompertz equation, the CH_4 production seemed to be more accurately described (R^2 = 0.987). Moreover, Kim et al. [90] highly recommended to use another modified Gompertz equation, namely the modified Gompertz model 2. The authors demonstrated that this model could successfully describe the progress of CH_4 generation of a substrate with diverse organic fractions. In detail, this model was developed by adding a secondary term reflecting the second lag time of slowly degradable matter into the modified Gompertz equation. As such, the modified Gompertz equation was rewritten as following:

$$G(t) = G_1 \times e^{-e^{\left(\frac{R_1 \times e}{G_1} \times (\lambda_1 - t) + 1\right)}} + G_2 \times e^{-e^{\left(\frac{R_2 \times e}{G_2} \times (\lambda_2 - t) + 1\right)}} \quad (9)$$

where G(t) is the cumulative CH_4 yield at time t ($mLCH_4/gVS_{added}$); G_1 and G_2 are the CH_4 production potential in the initial and secondary stages ($mLCH_4/gVS_{added}$); R_1 and R_2 are the maximum CH_4 production rate at the initial and secondary stages (mL/d); λ_1 and λ_1 are the duration of initial and secondary lag phases (d); t is the duration of digestion at which the corresponding cumulative CH_4 production is recorded (d).

The curve-fitting plot of these models and corresponding regression parameters are shown in Figure 16, Figure 17 and Table 15. In a comparison, results obtained from the modified Gompertz model showed the high goodness of fit (R^2 = 0.995). The estimated lag times derived from this model were 8.4 and 23.1 days for the more easily degradable and slowly degradable materials, respectively. Apart from a long suspension in methanogenesis, the low maximum CH_4 production rates were estimated at the initial and secondary stages (i.e. 5.0 ± 0.1 and 12.2 ± 4.8 $mLCH_4/gVS_{added}$.d). These observations implied that raw primary sludge possessed organic fractions showing low CH_4 production rates. The presence of slowly biodegradable portion in sewage sludge was confirmed by Luostarinen et al. [82] who found that the significant CH_4 yield was only attained after 10 – 15 days at the batch mode. The complexity of raw primary sludge used in this study was found. The different ultimate CH_4 yields was calculated from the modified Gompertz model 2 (Table 15).

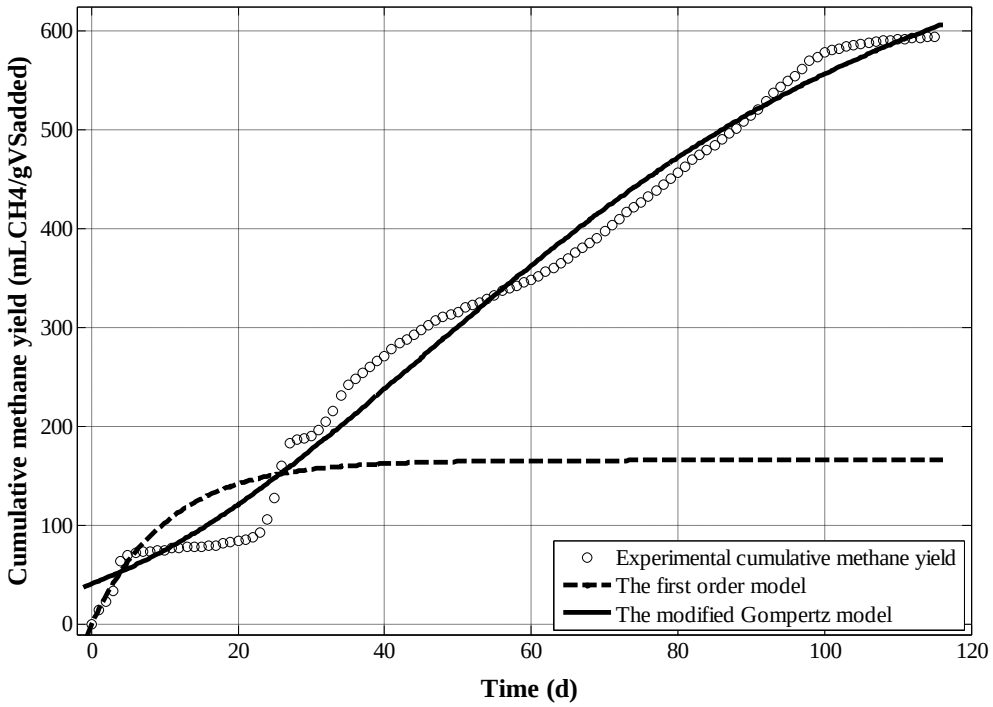


Figure 16. Plots of experimental cumulative CH_4 yield on VS basis and regression fitting curves of the first order model and modified Gompertz model applied for raw primary sludge.

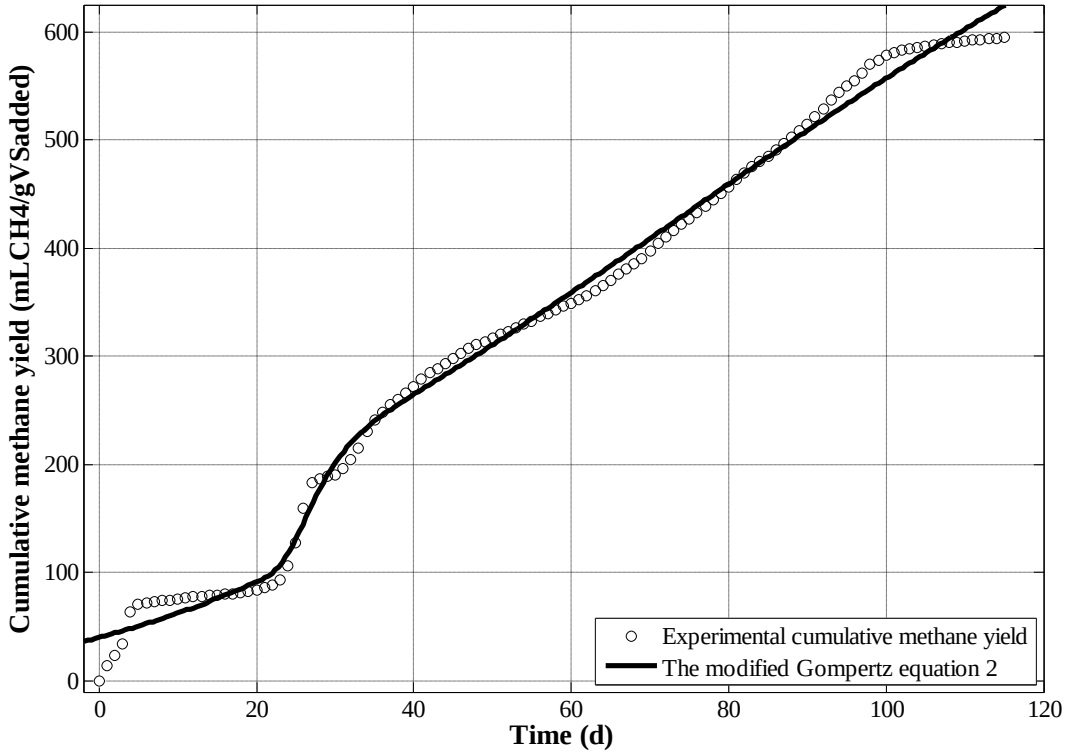


Figure 17. Plots of experimental cumulative CH_4 yield on VS basis and regression fitting curve of the modified Gompertz model 2 applied for raw primary sludge.

Nevertheless, the experimental and predicted ultimate CH_4 yield was of high values, reporting at 594.6 $mLCH_4/gVS_{added}$ (at digestion day 115) and 731.4 ± 38.5 $mLCH_4/gVS_{added}$ (from the modified Gompertz model), respectively. These values were comparatively higher with a range reported in the literature (Table 6). This discrepancy was probably due to various operational infrastructures, biogas collection systems, and calculation methods applied. It was here highlighted that raw primary sludge was one of potential substrate for CH_4 yield.

Stability in methanogenesis of raw primary sludge was observed during this batch test due to favourable anaerobic conditions (i.e. neutral pH and high buffer capacity). Experimental results and kinetic analysis data here gave evidences for the complexity of raw primary sludge in terms of organic fractions with different biodegradability. Although raw primary sludge were also identified to possess a great potential of CH_4 yield, low CH_4 production rate and long lag phase made it unfavourable for a well-established anaerobic digester. As such, the supplement of substrates rich in readily biodegradable part was prospectively considered to simulate the methanogenesis of raw primary sludge in particular and sewage sludge in general.

Table 15. Kinetic analysis of CH_4 production potential of raw primary sludge.

Models and results (at 95% confidential interval)	**Raw primary sludge**
Experimental cumulative CH_4 yield ($mLCH_4/gVS_{added}$)	594.6
Modified Gompertz model	
Lag phase - λ (d)	2.412
R^2	0.987
Maximum CH_4 production rate - R_m ($mLCH_4/gVS_{added}.d$)	6.3 ± 0.2
Predicted ultimate CH_4 yield ($mLCH_4/gVS_{added}$)	731.4 ± 38.5
Modified Gompertz model2	
Lag phase 1 – λ_1 (d)	8.438
Lag phase 2 – λ_2 (d)	23.12
R^2	0.995
Maximum CH_4 production rate – R_1 ($mLCH_4/gVS_{added}.d$)	5.0 ± 0.1
Maximum CH_4 production rate – R_2 ($mLCH_4/gVS_{added}.d$)	12.2 ± 4.8
Predicted ultimate CH_4 yield – G_1 ($mLCH_4/gVS_{added}$)	890.9 ± 109.8
Predicted ultimate CH_4 yield – G_2 ($mLCH_4/gVS_{added}$)	98.5 ± 17.2

4.2 Glycerol as a co-substrate

4.2.1 Characteristics of pure and crude glycerol

Three different types of glycerol (namely pure, biodiesel and BIA) were used in this investigation. Their key properties are summarised in Table 16.

In general, high solubility of glycerol in water made it readily bioaccessible to microorganisms. The high COD levels (1030 – 1140 g/L) of glycerol, moreover, indicated its high source of biodegradable carbon, the primary substrate for anaerobic microbes. These observations are in good agreement with that previously reported (Section 2.3.1).

Comparatively, crude glycerol investigated in this study had a similar range of glycerol content with that published in the literature (Table 16). A typical crude glycerol produced from homogeneous base-catalyzed transesterification possesses 50 – 60% of glycerol (Section 2.3.1). Thompson and He [150] reported that the purity of crude glycerol from various vegetable oils is from 60 – 70%. In another study, the purity of crude glycerol from seeds and beans was reported at between 78 – 84% [151].

Table 16. Physicochemical properties of pure and crude glycerol.

Properties	Pure	Biodiesel	BIA
Appearance	Clear	Amber	Pale brown
Odour	Odourless	Grain like odour	Mild odour
pH	6.7	4	8 – 9
Boiling point (°C)	290	>130	>130
Solubility in water	Highly soluble	Highly soluble	Highly soluble
Na content (mg/L)	0	40	16,939
K content (mg/L)	0	119	454
Specific gravity (at 25 °C)	1.26	1.22–1.24	1.25
Glycerol content (%)	≥ 99	~75-85	~50
COD (gO_2/L)	1048	1030	1140

BIA = BIA glycerol

Certain key physicochemical characteristics of glycerol can be related to their impurities. Specific gravity is one instance. It has been determined that the specific gravity of a 100% pure glycerol is about 1.26 at 25 °C. Of used glycerol, biodiesel and BIA glycerol had a lower specific gravity (1.22 – 1.25) probably due to the

presence some lighter constituents than glycerol, such as hydrocarbons and water [18, 150]. These impurities might be derived from biodiesel production. At the operational temperature (35 °C), it was indicated that their viscosity was proportionally increasing to their purity, with the highest viscosity being observed in pure glycerol, followed by biodiesel and BIA (Figure 18).

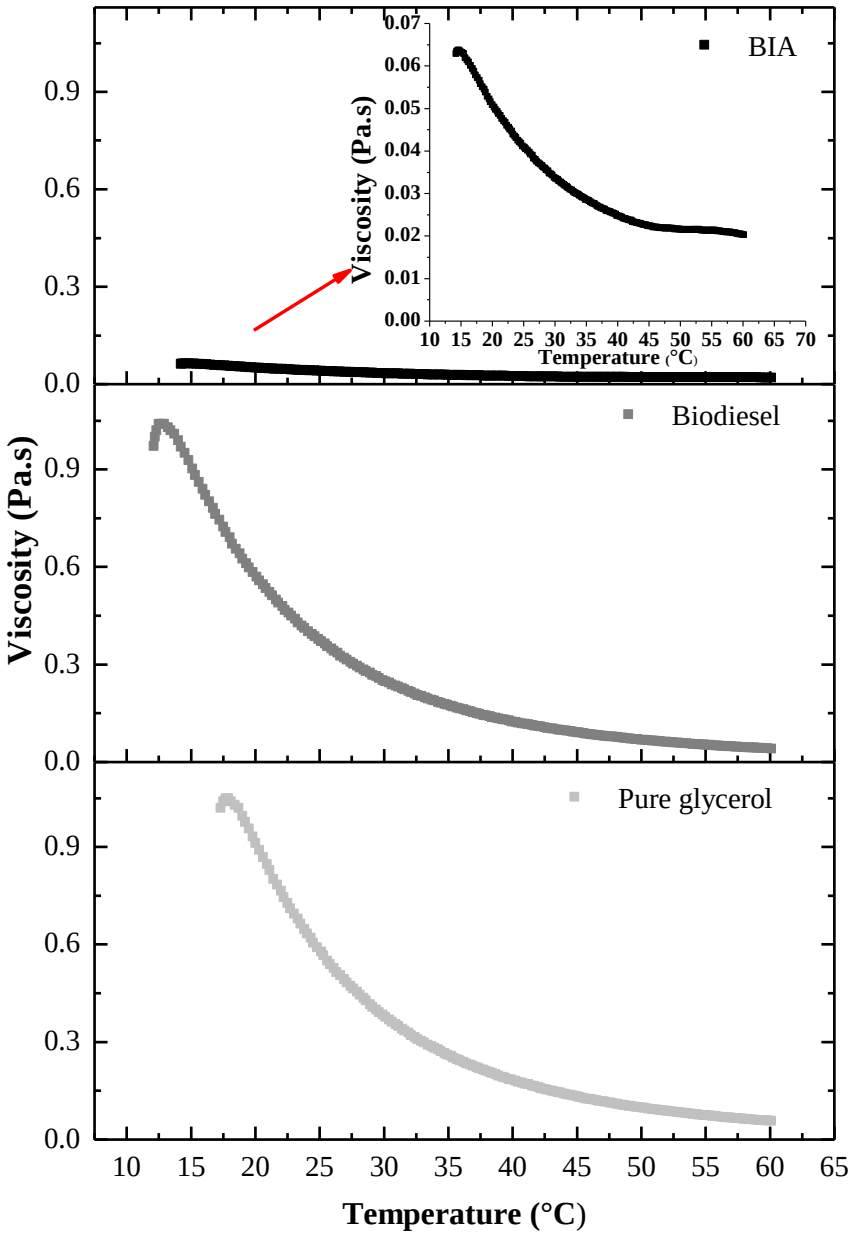


Figure 18. Viscosity of pure glycerol and two types of crude glycerol (biodiesel and BIA) as a function of temperature from 10 °C to 60 °C.

Another marginal difference between pure and crude forms of glycerol is a variety of elements. Sodium and potassium content were here reported at relative greater

quantities for BIA glycerol at 16939 and 454 mg/L respectively than for biodiesel glycerol. They are resulted from catalysts used in processing and neutralisation step [151]. Applying different biodiesel production processes and purifying approaches, together with various sources of feedstocks, therefore, brought out a wide range of purity of this versatile byproduct. Consequently, the presence of these impurities may lead to the lower CH_4 production (Section 2.3.2).

4.2.2 Variations of control parameters in the study of methanogenesis of glycerol

Glycerol was introduced into digested sludge at increasing percentages for evaluating the kinetic and potential of their CH_4 productions. Details of this batch test were described in Section 3.2.3. Characteristics of this seed (labelled as R0), compositions and some relevant features of sample mixtures are shown in Table 17. 0.25 and 0.5% each glycerol were mixed with this fresh inoculum to make up sample mixtures. They were labelled as P-0.25, P-0.5, BID-0.25, BID-0.5, BIA-0.25, and BIA-0.5 (with P, BID, and BIA denotes pure, biodiesel, and BIA glycerol, respectively).

It should be noted that the introduction of low quantity of glycerol into digested sludge had a negligible impact on all parameters, except for COD and total organic acids. Irrespectively of glycerol type, pH varied between 8.1 and 8.7. Likewise, there was a slight fluctuation among buffer capacity values of all sample mixtures. Compared to background values of digested sludge, including pH of 7.6 and alkalinity of 4186 mg$CaCO_3$/L, these obtained values were not significantly different. In this set-up, all BMP bottles were operated with favourite start-up conditions, including close to neutral pH and reasonably high alkalinity (ca from 3000 to 4000 mg$CaCO_3$/L). As such, it was expected that methanogenesis lasts longer to accomplish the ultimate CH_4yields.

Both TS and VS content in the sample mixtures experienced a slight increase as related with glycerol loads. In detail, background concentrations of TS and VS were 21.3 g/L and 13.6 g/L, lower than those of sample mixtures of 23.1 – 25.9 g/L and 15.2 – 17.3 g/L, correspondingly. Likewise, initial values of COD_s were proportional to increasing glycerol concentrations. The addition of 0.25% and 0.5% of glycerol considerably enhanced the organic matter concentrations, around four times in COD_s concentration (Table 17). The ratio between COD_s and COD_t, so-called COD yields, expressing the extent of solubilisation of samples. In the sample mixtures, this fraction was in the range of 3.8 – 18.5%, greater than that of digested sludge (3.5%).

Table 17. Key properties of sample mixtures of three types of glycerol and sludge.

	Characteristics	Sludge samples						
		Inoculum (DS)	**P-0.25**	**P-0.5**	**BID-0.25**	**BID-0.5**	**BIA-0.25**	**BIA-0.5**
	Types of glycerol	-	Pure	Pure	Biodiesel	Biodiesel	BIA	BIA
	Glycerol (% of DS)	-	0.25	0.5	0.25	0.5	0.25	0.5
Initial values	TS (g/L)	21.3	23.1	25.9	24.4	24.5	23.7	24.3
	VS (g/L)	13.6	15.3	17.3	16.1	16.3	15.2	16.0
	pH	7.6	8.7	8.6	8.7	8.1	8.7	8.7
	Alkalinity ($mgCaCO_3/L$)	4186	3521	3401	3928	3378	4331	3950
	COD_t (g/L)	22.4	23.3	25.8	23.9	26.0	23.0	27.1
	COD_s (g/L)	0.78	0.9	4.0	1.3	4.8	1.3	1.8
	Total organic acids (mg/L)	703.9	1468	1781	1941	1964	2412	2699
	COD_s/COD_t (%)	3.5	3.8	15.5	5.4	18.5	5.7	6.6
	NH_4^+-N (mg/L)	610	nd	nd	nd	nd	nd	nd
Final values	TS (g/L)	22.9	19.7	21.1	21.5	20.6	19.8	20.7
	VS (g/L)	13.4	11.5	13.0	13.2	12.9	12.1	12.7
	pH	7.7	8.1	8.1	8.0	8.2	8.2	8.2
	Alkalinity ($mgCaCO_3/L$)	5948	5023	5042	5140	5033	5316	5423
	COD_t (g/L)	21.7	17.0	19.2	22.4	19.5	17.4	16.7
	COD_s (g/L)	0.75	0.43	0.64	0.58	0.64	0.58	0.68
	Total organic acids (mg/L)	nd	892	817	799	920	1156	1232
	NH_4^+-N (mg/L)	nd			1015	1180	1171	1156
	VS removal (%)	nd	24.8	24.9	18.0	20.9	20.4	20.6
	TS removal (%)	nd	14.7	18.1	11.9	15.9	16.5	14.8
	COD_s removal (%)	nd	52.2	84.0	54.0	86.6	54.0	61.8

Digested sludge (DS), nd = no data; all data are expressed as mean values; P-0.25, P-0.5, BID-0.25, BID-0.5, BIA-0.25, and BIS-0.5 refer to mixtures of DS and pure (P), biodiesel (BID) and BIA glycerol at 0.25% and 0.5% each by volume, respectively

Simultaneously, the glycerol introduction increased the organic acid levels. This observation suggests the availability of organic acid content in three types of glycerol. As seen in Table 17, greater amount of organic acids was observed in crude than pure glycerol, referring to higher impurity of crude glycerol. It was pointed out in some studies that this constituent in residual glycerol could be derived from raw materials and inhibit bacterial activity [151]. From Figure 19, nonetheless, it can be seen that anaerobic digesters were expected to be constantly well performed without any risk of acid accumulation since the ratio of total organic acids and alkalinity was still within the satisfactory range (0.3 – 0.4).

Given that this anaerobic treatment process was launched with initial adequate control parameters as discussed above, the stability of the anaerobic conversion was able to be monitored in some extent by evaluating these parameters at the end. All final values are presented in Table 17.

The final pH values still laid within the range for normal growth of methanogens (ca 8). Furthermore, relatively higher values of total alkalinity at the end, from 5023 to 5948 mg$CaCO_3$/L. These values may implicate the stable performance of this AD process. Due to the fact that equilibrium of buffer capacity is created by ammonia nitrogen, organic acids, and CO_2 [143], higher buffer capacities after incubation could be elucidated by the production of ammonia nitrogen or by the consumption of organic acids. Higher ammonia nitrogen concentrations at the end, up to around 1000 mg/L, were a consequence of biodegradation of the nitrogen-containing matter. However, no clear relationship between ammonia nitrogen levels and load added as well as glycerol types was obtained. Hence, these nitrogen-containing materials were essentially originated from digested sludge but glycerol, a nitrogen-free substrate [152]. Additionally, there was no accumulation of organic acids regardless of any used glycerol, reflecting their effective degradation by anaerobic ecosystem. This indicated no suppression of methanogenic activity happened and the hydrolytic-acidogenic stage was successfully completed.

Correspondingly, all bottles showed a clear reduction of COD_s. As related to increasing glycerol concentrations, the COD_s removal increased with from around 50 to 80% each kind of glycerol (Table 17). Additionally, removal efficiencies of COD_t, TS, and VS were also achieved at lower extent. All mixtures at different glycerol types and concentrations showed the similar conversion performance of TS and VS, 11.9 – 18.5% of TS removal and 18.0 – 24.9% of VS removal. The values show

anaerobic digesters were healthily functioned at the methanogenesis step to consume soluble matters from hydrolytic-acidogenic step. Another indicator of sustainable working of current BMP bottles is the ratio of total organic acids and total alkalinity. In the current batch test, this ratio ranged between 0.15 and 0.2 at the end of digestion time, showing well-buffered systems (Figure 19).

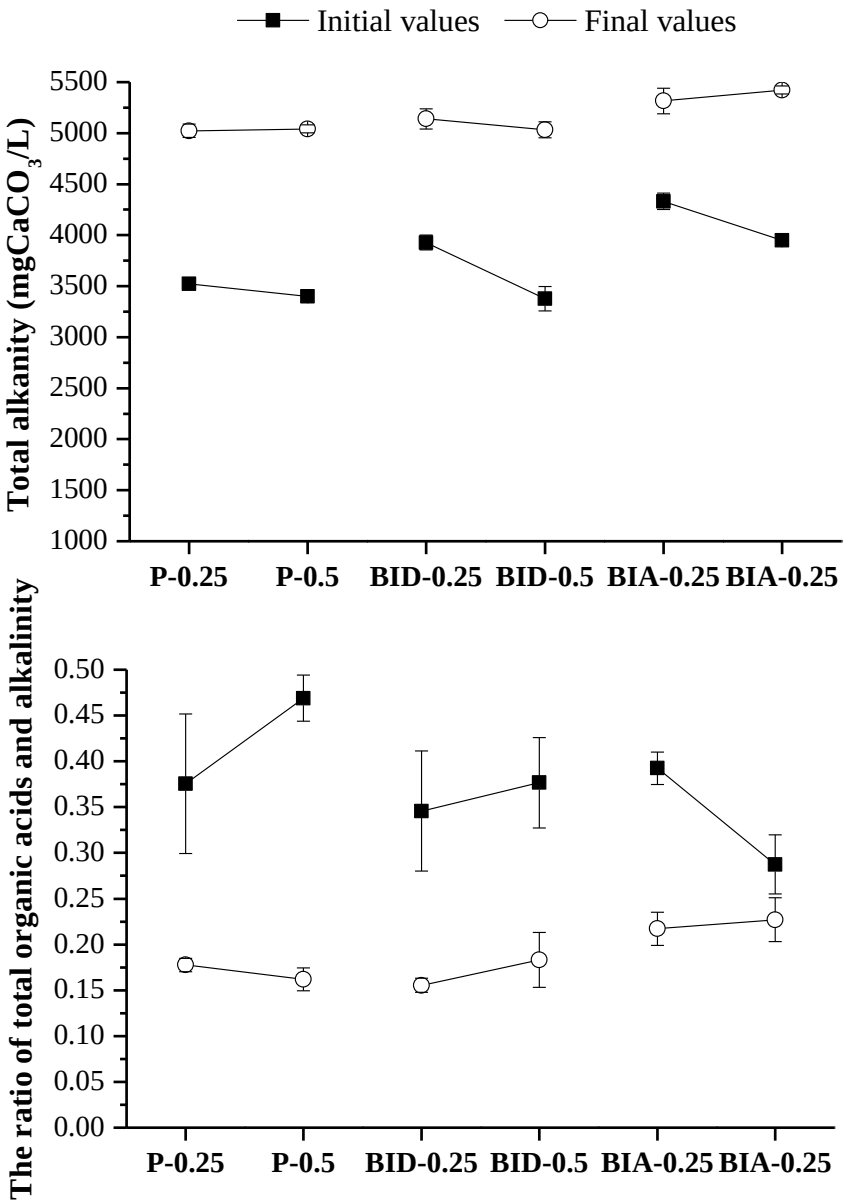


Figure 19. Variations of total alkalinity and ratios of total organic acids and total alkalinity as related to type and concentrations of glycerol; 0.25% and 0.5% addition of pure (P), biodiesel (BID) and BIA glycerol were labelled as P-0.25, P-0.5, BID-0.25, BID-0.5, BIA-0.25, and BIA-0.5 respectively; error bars refer to the standard deviation (n=2).

Considering all control parameters, 0.25% and 0.5% of each glycerol applied under current conditions investigated in the present work are still not beyond the limit concentration. This is a result of approaching a reasonable I/S ratio, ranging from 1.48 to 1.52 (gCOD/gVS) and from 1.49 to 1.59 (gCOD/gVS) respectively for 0.25% and 0.5% addition of glycerol.

4.2.3 Methanogenic conversion of glycerol

Figure 20illustrates the cumulative CH_4 yield as a function of digestion time with different types and ratios of glycerol to digested sludge. Data reported here proved that on average the usage of glycerol, a highly degradable carbon source, stimulated the anaerobic conversion. CH_4 production or substrate utilization rates of all glycerol treatments mostly showed peaks for the first few days with the highest rate of 0.25% pure glycerol (193.4 $mLCH_4$/gCOD.d) at the first day. This indicated that anaerobic microbes were quickly able to digest the glycerol. It could be ascribed to easily biodegradability of glycerol.

All types of glycerol had the similar fashion of CH_4 production, which increased until around day 10, beyond that a gradual stable trend was reached (Figure 20). However, two contrasting trends in rates of generating CH_4were obtained when anaerobically treating different types of glycerol. Pure and BIA glycerol showed higher CH_4 potential at lower concentration (0.25%). The time for acclimatisation could be one of the reasons behind this behaviour. The longer lag phases were observed in 0.5% compared to that in 0.25% addition of pure and BIA glycerol. This is due to the fact that bacteria in digested sludge had no previous exposure to glycerol. On the contrary, higher CH_4 production of the digester treating 0.5% than 0.25% biodiesel glycerol was obtained (Figure 20). Residual glycerol from biodiesel production process had a remarkable methanol content of 8 – 12% by weight [18, 96, 150]. Under anaerobic condition, this constituent is directly converted into CH_4. Accordingly, the more methanol biodiesel glycerol has, the more methanogenic activity is accelerated.

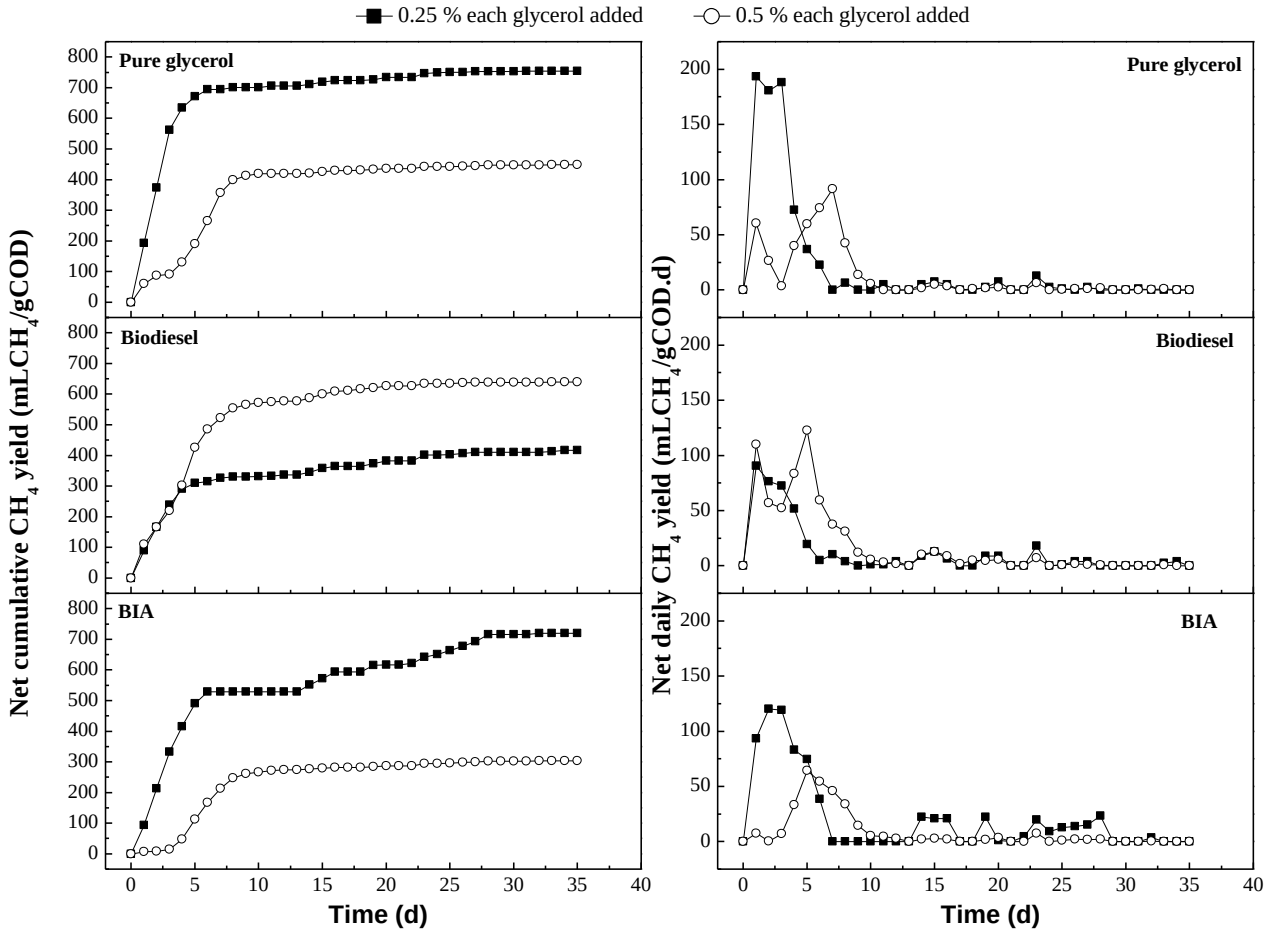


Figure 20. Cumulative CH_4 yield and CH_4 production rate of three different types of glycerol, namely pure (P), biodiesel (BID) and BIA, at increasing concentrations of 0.25% and 0.5%, respectively labelled as P-0.25, P-0.5, BID-0.25, BID-0.5, BIA-0.25, and BIA-0.5.

Like the CH_4 production rates, similar observations were obtained with cumulative CH_4yield for all types of glycerol. On average, these experimental values were calculated at 754.5, 449.1, 416.8, 639.5, 720.5, and 303.5 $mLCH_4$/gCOD respectively for P-0.25, P-0.5, BID-0.25, BID-0.5, BIA-0.25, and BIA-0.5, at day 35. Data here revealed the comparatively higher performance of BMP bottles to the values published. Siles López et al. [133] reported cumulative CH_4 yields of pre-treated glycerol achieved the highest point at the lowest loading. They stated that the acidified glycerol treated with granular sludge exhibited the highest CH_4 yield of 323 $mLCH_4$/gCOD. The lowest CH_4 yield observed in BIA treatment is in line with these values. Meanwhile, there is a good agreement between the CH_4 yield of 0.25% pure glycerol and that value reported by Amon et al. [153].

As expected, the higher purity of glycerol, the greater CH_4 production of BMP bottles. The highest CH_4 potential (754.5 $mLCH_4$/gCOD) was reached with the pure form of glycerol even at lower concentration, meanwhile the treatment of 0.5% BIA resulted in the lowest performance in methanogenesis (303.5 $mLCH_4$/gCOD). There was, therefore, a correlation of CH_4 yield with the impurity of glycerol. Among substrates, BIA had the lowest pure glycerol and the highest quantity of sodium (16.9 gNa/L), negatively affecting the methanogenesis of this glycerol. Inhibition concentrations of sodium have been reported widely in the literature [138, 154]. Under anaerobic condition, biomass activity and metabolism of anaerobic microbes could be readily interfered and then inhibited by high sodium concentration. In the biological sense, this phenomenon could be explained by the participation of Na^+ in channels on cell membrane and some metabolic steps. McCarty et al. [138] investigated the effect of sodium concentrations on methanogens, indicating that moderate to strong inhibition resulted from using 3.5 – 5.5 gNa/L and 8 gNa/L, respectively. For anaerobic granular biomass, Rinzema et al. [154] reported the decrease of10, 50, and 100% methanogenic activity was caused by sodium at concentrations of 5, 10 and 14 g/L, respectively, under mesophilic temperature and neutral pH.

Plots and kinetic coefficients of the first order and modified Gompertz models are presented in Figure 21, Figure 22, and Table 18. The soundness of kinetic models depends on the nature of compounds. It was stated that the first order kinetic model was suitable for a very simple substrate [155] while the more complex substrate

mixture was successfully monitored by using the modified Gompertz model [90]. The complexity of feedstocks according to Kim et al. [90] was due to the mixing of food waste and sewage sludge. To obtain higher correlation efficiencies, the authors tested a modified Gompertz model. In this study, data confirmed the fitness of both kinetic models to CH_4 generation of pure and biodiesel glycerol with relative high correlation (>91%). Results of these kinetic studies showed the short time taken for initializing the CH_4 generation. Regarding BIA, the first order kinetic model is better fitting to the trend of CH_4 production of 0.25% BIA. Alternatively, the modified Gompertz model showed higher goodness of fit in predicting the methanogenesis of 0.5% BIA. It could be due to this model could not reflect the lag phase of batch tests properly [90]. Longer lag phase in BMP bottles treating 0.5% BIA was probably caused by its higher concentration of impurities compared to that of bottles with 0.25% BIA.

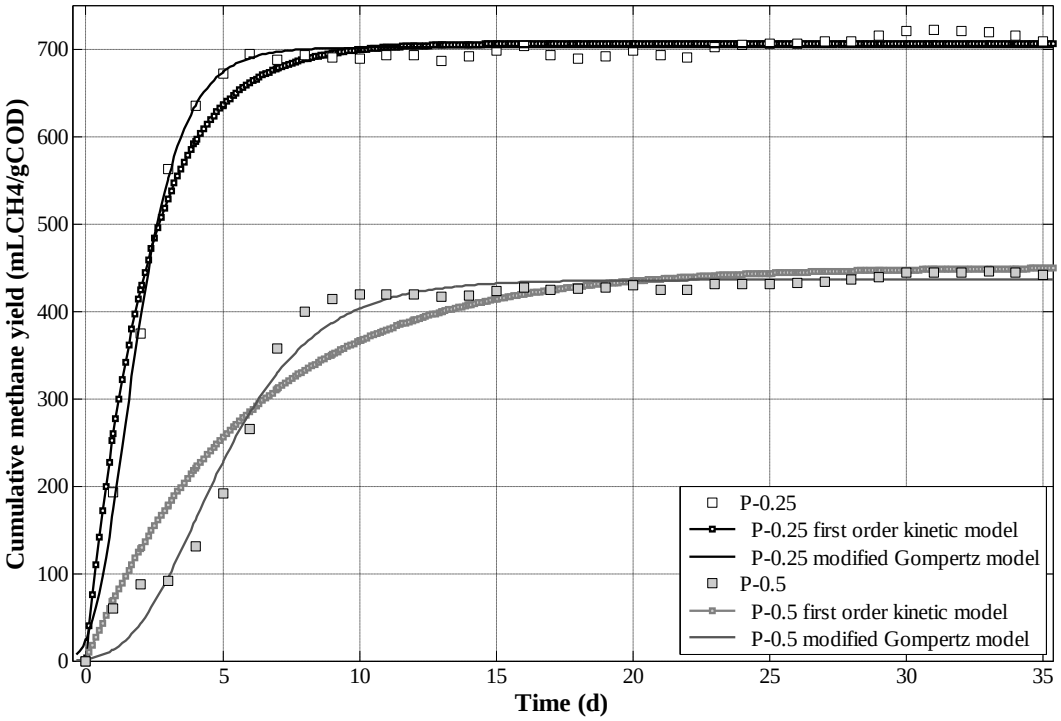
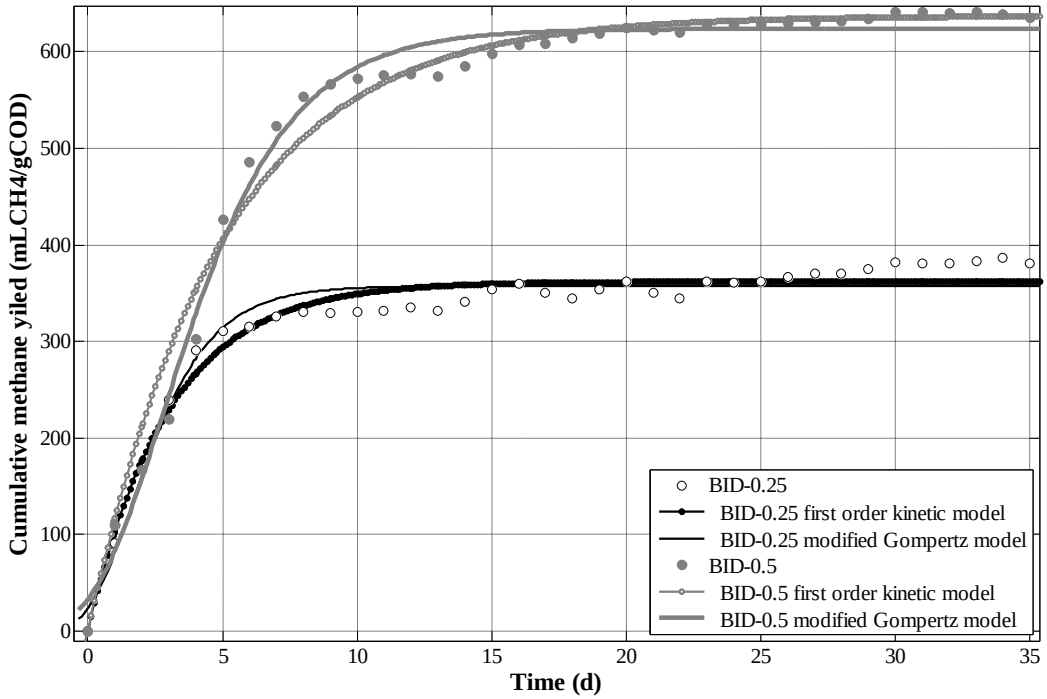


Figure 21. Plots of cumulative CH_4 yields on COD basis and regression fitting curves of the first order and modified Gompertz models of pure glycerol at two concentrations of 0.25% (P-0.25) and 0.5% (P-0.5).

a)



b)

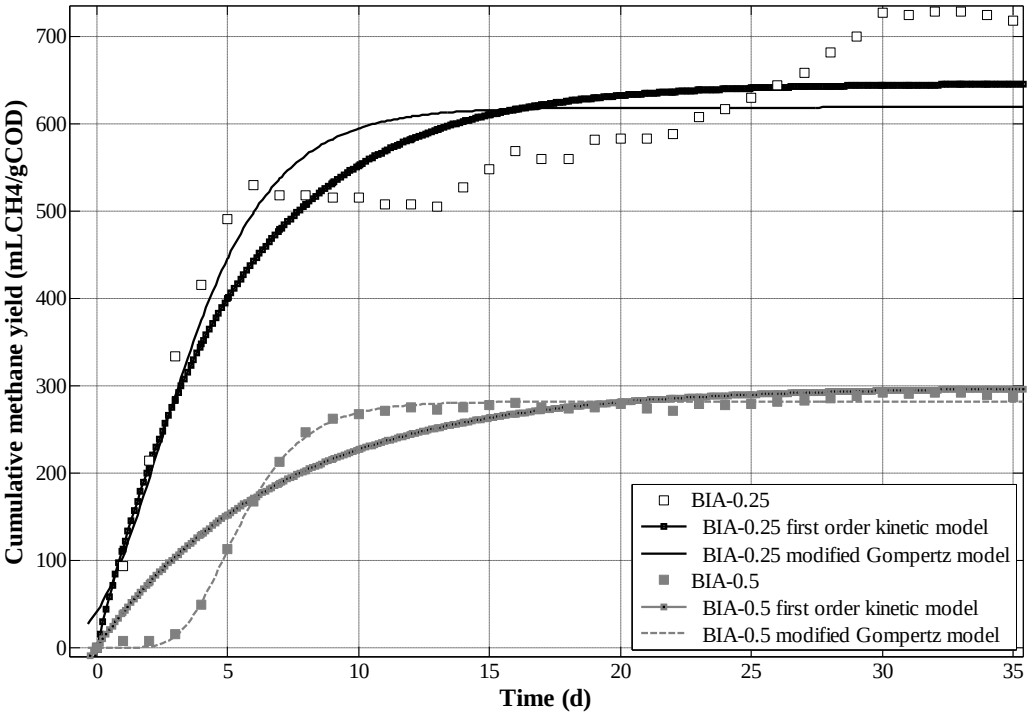


Figure 22. Plots of cumulative CH_4 yields on COD basis and regression fitting curves using the first order and modified Gompertz models of a) biodiesel (BID) and b) BIA glycerol at 0.25% and 0.5% concentrations, respectively labelled as BID-0.25, BID-0.5, BIA-0.25 and BIA-0.5.

Theoretical CH_4 yield of pure glycerol used in this experiment is calculated at approximately 428 $mLCH_4$/gCOD using Buswell equation below [156]:

$$C_3H_5(OH)_3 \rightarrow 1.75CH_4 + 1.25CO_2 + 0.5H_2O \quad (10)$$

by taking the specific gravity and COD values of pure glycerol of 1.26 and 1,148 gCOD/L, respectively, into account. Data obtained from experimental and predicted kinetics showed an excess of CH_4 yields compared to the theoretical value in case of 0.25% pure glycerol applied (i.e. more than 700 $mLCH_4$/gCOD). Similar observations were reported in a study on anaerobic co-digesting of glycerol with sewage sludge [19]. The authors attributed this greater experimental CH_4 yield to active biomass. It was clear that the growth of anaerobic microbes, particularly methanogens, were simulated by such readily degradable substrates as glycerol. Meanwhile, the proximate in values of theoretical and practical CH_4 yields when treating 0.5% pure glycerol was found.

Table 18. Kinetic analysis of CH_4 production during the batch test of BMP of glycerol.

Models and kinetic parameters (at 95% confidential intervals)	**Pure glycerol**		**Biodiesel glycerol**		**BIA glycerol**	
	0.25%	**0.5%**	**0.25%**	**0.5%**	**0.25%**	**0.5%**
Experimental cumulative CH_4 yield ($mLCH_4$/gCOD)	754.5	449.1	416.8	639.5	720.5	303.5
First order kinetic model						
First order rate constant – k (1/d)	0.410	0.163	0.261	0.200	0.189	0.131
R^2	0.982	0.934	0.941	0.983	0.921	0.915
Predicted cumulative CH_4 yield – G_o ($mLCH_4$/gCOD)	738.5 ± 8.7	458.2 ± 18.6	390.4 ± 10.0	640.6 ± 11.0	668.4 ± 24.6	314.2 ± 19.6
Modified Gompertz model						
Lag phase – λ (d)	0.154	1.558	0.000	0.185	0.000	3.057
R^2	0.985	0.976	0.913	0.988	0.879	0.993
Maximum CH_4 production rate – R_m ($mLCH_4$/gCOD.d)	208.3 ± 26.4	66.1 ± 9.0	72.5 ± 10.7	86.4 ± 8.2	93.6 ± 15.4	57.0 ± 6.3
Predicted cumulative CH_4 yield – G_o ($mLCH_4$/gCOD)	732.1 ± 7.8	442.6 ± 7.7	381.4 ± 11.2	627.0 ± 7.8	642.2 ± 24.9	292.9 ± 3.6

On basis of data of control parameters as related to glycerol types and concentrations suggests, the introduction of 0.25% and 0.5% of three types of glycerol still

demonstrated the adequate function of both hydrolytic-acidogenetic and methanogenetic bacteria. On the other hand, the profile of both experimental and predicted CH_4 yields implicated an obvious dependence of CH_4 yields of glycerol on its degree of impurities (i.e. sodium concentration). It could be concluded that glycerol could be considered as a feasible feedstock for the AD process owning to their high CH_4 potential. They might be potentially used as a high-yield co-substrate for enhancing the anaerobic treatment of low-carbon substrates.

4.3 Summary

In this batch test, the potential of CH_4 yields and process stability of the anaerobic conversion of each sewage sludge and glycerol was tested with different buffer concentrations and I/S ratios.

In terms of system stability, data from experimental observations indicated methanogens was favoured by the buffer ($NaHCO_3$) supplement. Compared to the non-buffed bottles, higher performance in producing CH_4 was recorded in bottles with addition of 15 and 30 mM $NaHCO_3$. Lag times estimated from the first-order and modified Gompertz models gave evidence of such stimulation of CH_4 conversion by buffer addition. Buffer concentrations applied in this study was, however, still not adequate for stable methanogenesis, possibly due to low inoculum. An increase of I/S ratio from 1/9 to 1/1 led to the stability in the methanogenesis of raw primary sludge due to favourable anaerobic conditions (i.e. neutral pH and high alkalinity), implying an important role of I/S ratio. In this steady state, results of CH_4 yield from experimental data and kinetic analysis demonstrated a great CH_4potential of raw primary sludge. However, low CH_4 production rate observed here suggested a need of readily biodegradable substrate in order to accelerate the methanogenesis of raw primary sludge in particular and sewage sludge in general.

Another series of BMP tests of glycerol, which has been stated a potential substrate for anaerobic digestion, was carried out with the adequate inoculum supplementation. Evaluation of control parameters among three types of glycerol at different concentrations showed a stable function of anaerobic conversion. It could be expected from their high experimental and predicted CH_4 yields that glycerol could be a feasible co-substrate for sewage sludge.

5 ANAEROBIC CO-DIGESTION OF SEWAGE SLUDGE AND GLYCEROL

A series of anaerobic co-digestion batch tests was performed using raw primary sludge and glycerol as a co-substrate. It is hypothesized that the addition of glycerol increases the carbon content of the feed mixtures, resulting in higher CH_4 yield. The effects of glycerol and buffer ($NaHCO_3$) addition on system stability were simultaneously assessed.

5.1 Characteristics of mixtures of sewage sludge and glycerol

The feeds, which was the mixtures of raw primary sludge and glycerol with the supplement of buffer ($NaHCO_3$) were characterised for their key characteristics in Table 19. This table also presents relevant properties of the seed, or digested sludge, and other sludge samples of raw primary sludge with and without supplied $NaHCO_3$ for comparison. Experimental procedure in this batch test was stated in Section 3.2.3.1. The mixtures of DS and RS at ratio of 1/9 (R-0) was supplemented with 15 mM and 30 mM $NaHCO_3$, one of which was then introduced with 0.5% and 1.0% glycerol, respectively, labelled as R-15-0.5, R-15-1.0, R-30-0.5, and R-30-1.0. Reference bottles containing increasing buffer concentrations and no glycerol were also utilised for co-digestion evaluation. They were R-0, R-15 and R-30, characteristics of which were discussed in Section 4.1.2.

Similar changes in TS and VS content were observed in BMP bottles supplemented with $NaHCO_3$ and glycerol. A slightly increase of VS and TS content in glycerol-added BMP mixtures (i.e. 0.5% and 1.0% pyre glycerol) with buffer compared to those adding only buffer (i.e. 15 and 30 mM $NaHCO_3$). Consequently, when VS/TS ratios were evaluated, no variation among these mixtures was observed, between 80.4% and 88.4% (Table 19).

There was no clear effect of the supplement of glycerol on both pH and alkalinity. Changes of pH of buffered bottles with glycerol added and that of buffered bottles including no glycerol was negligible irrespectively of glycerol concentrations (Table 19). At 15 mM $NaHCO_3$ added, pH were 6.6 without glycerol addition and changed to 6.5 after introducing 0.5% and 1.0% of glycerol. Correspondingly, these values were 6.9 and 6.8 in case of 30 mM $NaHCO_3$. Compared to pH variation, alkalinity values experienced the same fashion (Figure 23), demonstrating only $NaHCO_3$ proved its effect on enhancing buffer capacities of co-digestion mixtures as expected.

Table 19. Physical and biochemical characteristics of sample mixtures in the anaerobic co-digestion test.

	Characteristics	Sample mixtures							
		Inoculum (DS)	**R-0**	**R-15**	**R-15-0.5**	**R-15-1.0**	**R-30**	**R-30-0.5**	**R-30-1.0**
Content	I/S (by volume)	nd	1/9	1/9	1/9	1/9	1/9	1/9	1/9
	Glycerol (% of RS volume)	nd	Nd	nd	0.5	1.0	nd	0.5	1.0
	$NaHCO_3$ (mM)	nd	Nd	15	15	15	30	30	30
Initial values	TS (g/L)	21.3	24.1	27.8	34.4	34.0	31.5	34.0	31.9
	VS (g/L)	13.6	21.3	23.1	29.5	29.0	25.3	27.9	26.8
	VS/TS (%)	63.8	88.4	83.1	85.8	85.3	80.3	82.1	84.0
	pH	7.6	6.2	6.6	6.5	6.5	6.9	6.8	6.9
	Alkalinity ($mgCaCO_3/L$)	4186	1229	1952	1959	2090	2929	2709	2490
	COD_t (g/L)	22.4	31.4	31.7	34.5	37.3	29.7	37.8	39.9
	COD_s (g/L)	0.78	15.5	2.2	9.7	13.6	2.2	9.1	15.5
	COD_s/COD_t (%)	3.5	6.4	6.9	28.2	24.0	7.4	36.4	38.9
Final values	TS (g/L)	22.9	17.7	17.0	27.5	25.9	18.3	24.2	26.8
	VS (g/L)	13.4	14.0	12.6	22.4	20.8	12.2	17.8	21.1
	VS/TS (%)	58.5	79.1	74.1	81.5	80.3	66.7	73.6	78.7
	pH	7.7	4.4	4.5	4.4	4.4	4.7	4.6	4.9
	Alkalinity ($mgCaCO_3/L$)	5948	605	930	837	930	1442	1116	1758
	COD_t (g/L)	21.7	33.5	31.9	45.9	34.3	30.6	30.7	35.3
	COD_s (g/L)	0.75	10.8	12.1	13.7	14.0	12.6	15.0	16.4
	COD_s/COD_t (%)	3.5	32.2	37.9	29.8	40.8	41.2	48.9	46.2
	VS removal (%)	nd	34.1	45.7	24.0	28.3	51.5	34.5	21.0

Digested sludge (DS); raw primary sludge (RS); nd = no data, all data are expressed as mean values; R-15-0.5, R-15-1.0, R-30-0.5, R-30-1.0 refer to the sludge mixture (R-0) supplemented with 15 (R-15) and 30 mM $NaHCO_3$ (R-30), and introduced with 0.5% and 1.0% glycerol each, respectively

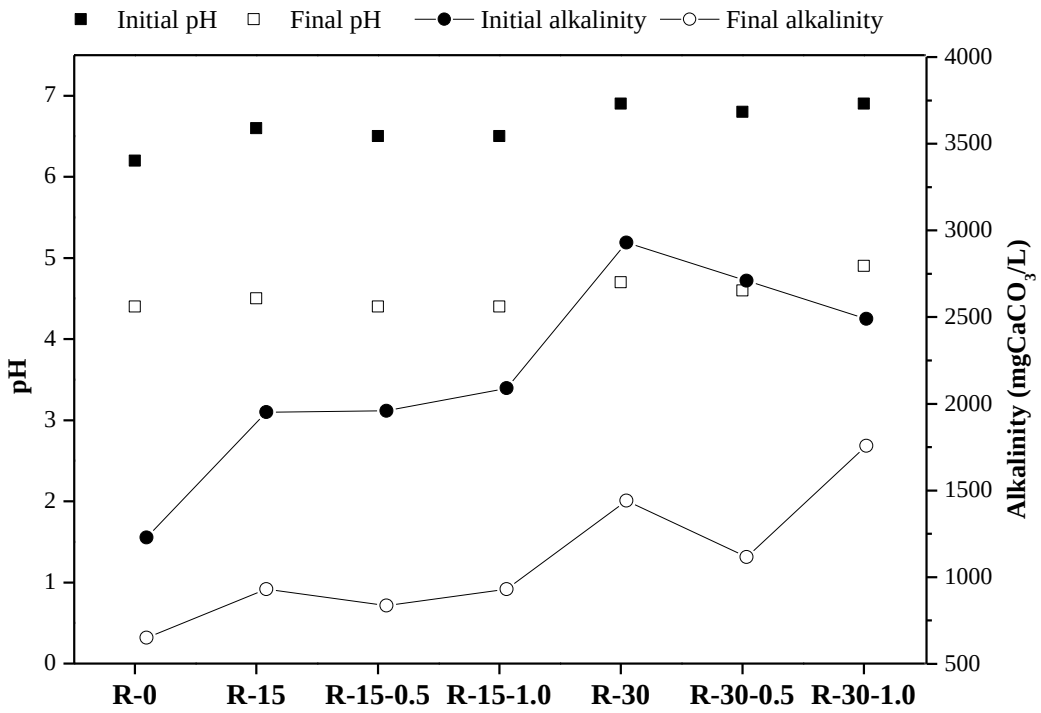


Figure 23. Values of pH and alkalinity as related to the glycerol and buffer addition before and after experimental time; R-15-0.5, R-15-1.0, R-30-0.5, R-30-1.0 refer to the mixture of 10% digested sludge and 90% raw primary sludge by volume (R-0) supplemented with 15 (R-15) and 30 mM $NaHCO_3$ (R-30), and introduced with 0.5% and 1.0% glycerol each, respectively.

As discussed previously (Section4.1.2), 15 and 30 mM $NaHCO_3$had an important role in well buffering the AD process by increasing pH and alkalinity levels without causing any change of the other parameters. As such, the start-up of this co-digestion test was facilitated with close to neutral pH and relatively high alkalinity due to the $NaHCO_3$ supplementation.

Apparently, increase of the biodegradable organic content for BMP bottles weremainly contributed by organic matter of glycerol. Whilst the glycerol supplementation caused a slight increase of TS, VS, and COD_t, the liquid part of all co-digestion mixtures had a considerably greater COD_s compared to the mono-digestion bottles (Table 19). Figure 24 shows thatCOD_s concentration increased proportional to glycerol addition (i.e. 0.5 and 1.0% v/v glycerol added) at any buffer concentrations (i.e. 15 and 30 mM $NaHCO_3$). Soluble organic fraction (COD_s) values of mixtures were around four times and seven times higher respectively after adding 0.5 and 1.0% glycerol than mixtures without glycerol addition. Accordingly, there

was a corresponding increase in the ratio of COD_s over COD_t concentrations with the addition of glycerol, indicating an improved extent of solubilisation in anaerobic co-digesters. This observation was expected due to the high solubility and purity of glycerol. There was a good agreement between data obtained here and that widely reported elsewhere. Several studies have noticed that the introduction of glycerol had a positive impact on the organic matter in sample mixtures[19, 20, 53, 152, 153, 157-159]. According to Wohlgemut et al. [157], the COD load was gradually improved by introducing increasing percentage of both pure and crude glycerol (i.e. 0.5, 1, 2 and 4% by weight) into reactors treating hog manure. A steady increase in COD level was also stated by Astals et al. [53] when using crude glycerol as a co-fermentation additive of pig manure. Siles et al. [20] carried out an increase COD load from 185 ± 5 $gCOD_s/L$ to 300 ± 5 $gCOD_s/L$ in the water phase by adding 15% glycerol (by volume) to wastewater from biodiesel production. An even eight times higher in COD_s concentration was also observed in co-digestion bottles (3% crude glycerol added) compared to reference digester [152, 158].

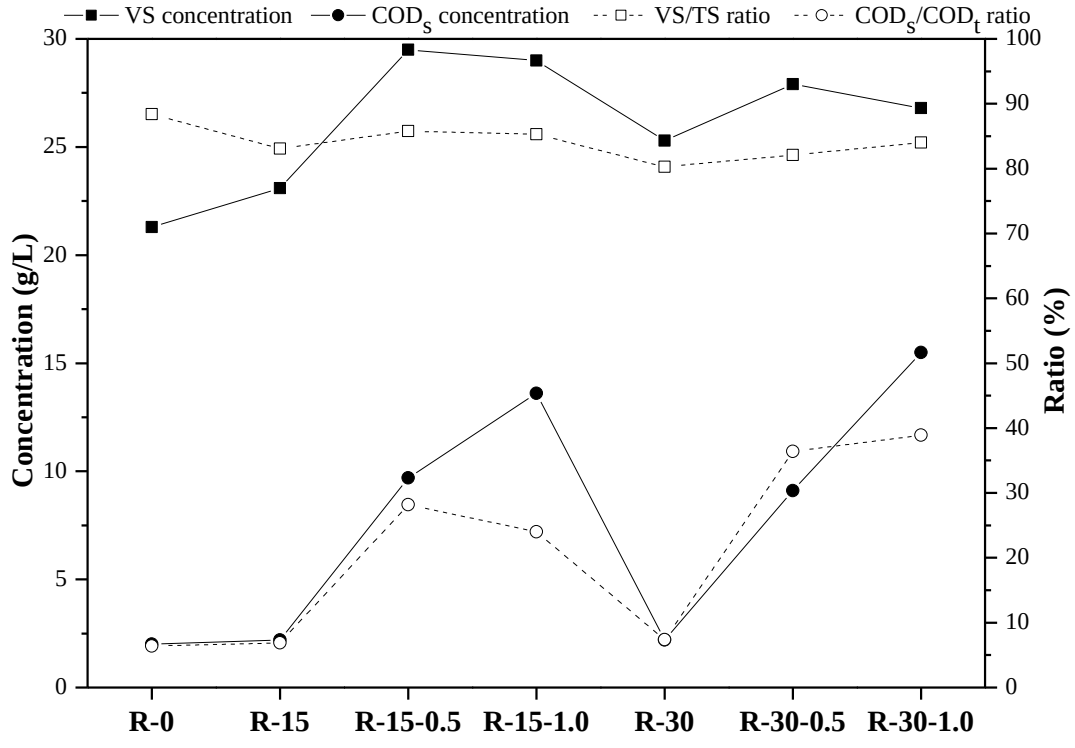


Figure 24. Changes of VS, COD_s, and ratios of VS/TS and COD_s/COD_t in relation with glycerol and buffer addition; R-15-0.5, R-15-1.0, R-30-0.5, R-30-1.0 refer to the mixture of 10% digested sludge and 90% raw primary sludge by volume (R-0)

supplemented with 15 (R-15) and 30 mM $NaHCO_3$ (R-30), and introduced with 0.5% and 1.0% glycerol each, respectively.

Glycerol is highly soluble in water while $NaHCO_3$ addition improves the buffer capacity. Hence, they were expected to create favourable conditions for anaerobic conversion in terms of system stability and CH_4 production performance.

5.2 Removal of volatile solids and chemical oxygen demand

The characteristics of digestates in this batch test are presented in Table 19. Stability and performance of single-digestion and co-digestion with glycerol of raw primary sludge were evaluated via the variations of VS and COD.

In comparison to the feed, after anaerobic metabolism, the digestate of all BMP bottles have a low pH value in the range of 4.5 – 4.9. This acidic pH range, which could be interpreted as a build-up of organic acids, can induce the system instability [142, 160-162]. It is not surprising that buffer capacities were deteriorated in all digestates (Figure 23). Therefore, $NaHCO_3$ concentrations in this study unsatisfactorily buffered the AD process. Buffer supplementation, nevertheless, still exhibited its function since significant differences of alkalinity levels were found with different $NaHCO_3$ concentrations added to the system. Available alkalinity values were of 837 – 930 $mgCaCO_3/L$ at 15 mM $NaHCO_3$ introduced while bottles buffered with 30 mM $NaHCO_3$ had alkalinity more than 1000 $mgCaCO_3/L$.

Co-digestion bottles exhibited lower VS content removal than the reference bottles. Final VS concentrations of BMP bottles with glycerol were significantly higher than those of co-digestion BMP bottles (Figure 25). At 15 mM added buffer, in particular, final VS values were 12.6 g/L, 22.4 g/L, and 20.8 g/L as 0%, 0.5%, and 1.0% addition of glycerol. These observations could be elucidated by two factors. First, the supplementary organic carbon source from glycerol simulated the biomass growth related to an increase in VS content. This is also stated by Ma et al. [159] who found a VS increase of 3 g/L in the UASB reactor with the glycerol supplementation after 33 digestion days. In a more recent study, Fountoulakis et al. [19] reported that an increase of VS concentration, from 17.9 ± 0.8 g/L to 23.7 ± 0.7 g/L after adding glycerol was a results of biomass growth stimulation. Second, some particulate organic matter remained in the digestate of co-digestion bottles could be another reason. This conclusion was clearly confirmed with data of VS/TS ratios, ranging from 70% to 80%, which generally were of 60 – 70% in a typical anaerobic digester.

Therefore, the hydrolytic bacteria in reference bottles better functioned. Astals et al. [158] had the same observation and explained by the fact that bacteria in co-digestion reactors favourably utilised glycerol, while biomass might digest particulate organic content in raw primary sludge in the absence of glycerol.

The variation of VS removed after digestion in relation to the addition of glycerol isshown in Figure 25. There was no notable difference of VS removal efficiencies between buffered and non-buffered BMP bottles. A decreasing trend of VS destruction was found when BMP bottles were fed with increasing glycerol concentration. Of BMP bottles buffered with 30 mM $NaHCO_3$, the highest performance of degrading VS was achieved at 51.5% in the bottle R-15, followed by 34.5% and 21.0% in R-30-0.5 and R-30-1.0 respectively. Likewise, at 15 mM $NaHCO_3$, bottles with 0%, 0.5%, and 1.0% glycerol added degraded 45.7%, 24.0%, and 28.3% of available VS correspondingly. The VS elimination was one of indicators of well hydrolysing particulate organic matter in substrate.

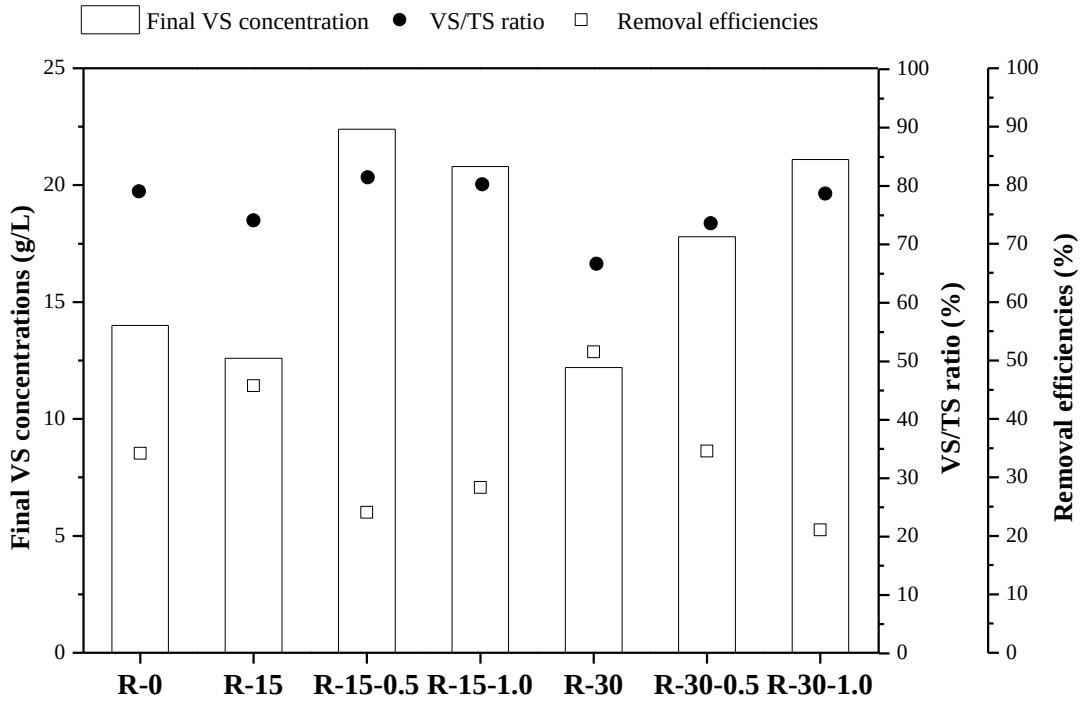


Figure 25. Profile of solid content, including final VS concentrations, VS/TS ratio, VS removal efficiency at the end of digestion; R-15-0.5, R-15-1.0, R-30-0.5, R-30-1.0 refer to the mixture of 10% digested sludge and 90% raw primary sludge by volume (R-0) supplemented with 15 (R-15) and 30 mM $NaHCO_3$ (R-30), and introduced with 0.5% and 1.0% glycerol each, respectively.

In addition, a considerable increase of COD_s after digestion process, a representative of soluble matter, was observed (Figure 26). Accumulation of VFAs, an intermediate product during anaerobic conversion, was considered as a reason owning to an acidic pH of digestates. A conjunction of VS destruction and VFA build-up here pointed out an adequate hydrolysis and acidogenesis, and simultaneously a visible suspension of methanogens.

When physicochemical features of influents and effluents of co-digestion and reference BMP bottles are taken into account, a reduction in pH to acidic values (ca 4 – 5), a destruction of buffer capacities (ca 1000 $mgCaCO_3/L$), and a considerable increase in COD_s (generally from VFA accumulation) were observed. Data reported here suggested an obvious instability of anaerobic system; an inadequate efficiency with regard of VS degradation was a consequence. Simultaneously, it is possible to relate this destabilisation to CH_4 formation although hydrolysis and acidogenesis were considered well-functioned.

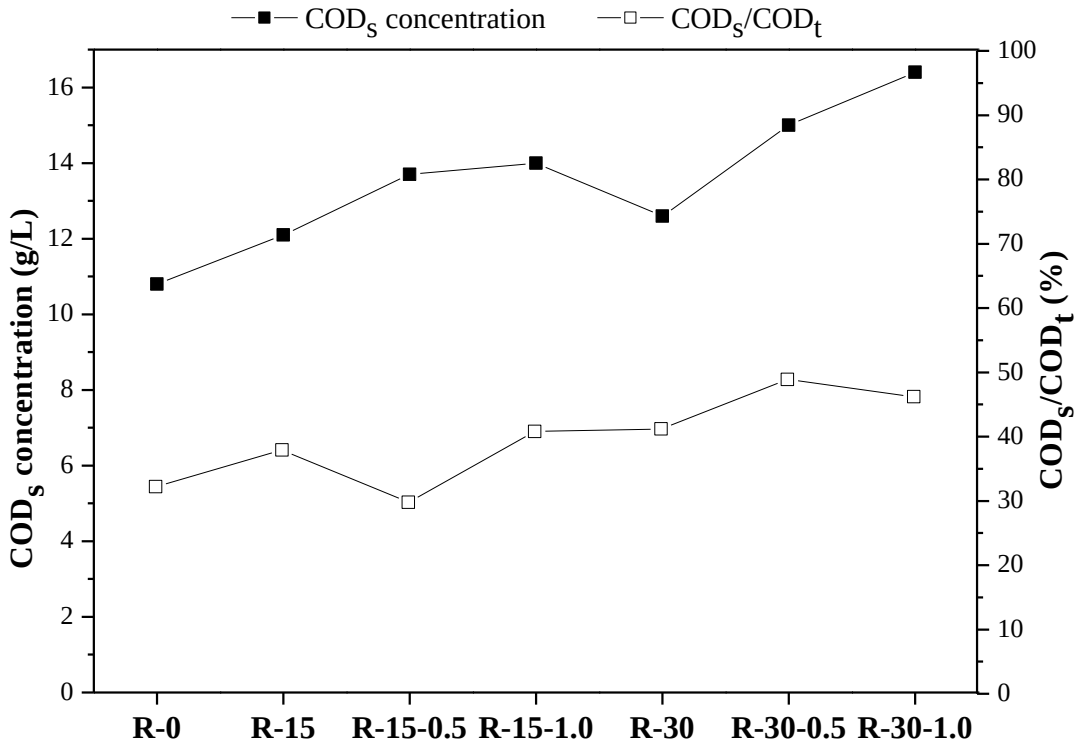


Figure 26. COD_s concentrations and COD_s/COD_t ratios in digestates; R-15-0.5, R-15-1.0, R-30-0.5, R-30-1.0 refer to the mixture of 10% digested sludge and 90% raw primary sludge by volume (R-0) supplemented with 15 (R-15) and 30 mM $NaHCO_3$ (R-30), and introduced with 0.5% and 1.0% glycerol each, respectively.

5.3 Methane potential of co-digestion mixture of sewage sludge and glycerol

The extent and yield of CH_4 production were experimentally observed during the anaerobic co-digestion of raw primary sludge with glycerol in a comparison with reference bottles. Their estimated results from two kinetic models were also included in order to predict and evaluate the effects of glycerol on the CH_4 conversion of raw primary sludge.

CH_4 production in all BMP bottles reached its peak at the first day of digestion time (Figure 27). Higher peaks of CH_4 production were observed with the co-digestion bottles compared to the reference bottles (R-0) containing raw primary sludge only. On average, with15 mM $NaHCO_3$ supplement, the highest CH_4 production rates were reached at 267.5 and 255 $mLCH_4$/d for R-15-0.5 and R-15-1.0, respectively, while 175 $mLCH_4$/d was the highest rate of methanogenesis from R-0. This indicated that bacteria immediately consumed glycerol. Thus, the addition of glycerol in the feed accelerated the hydrolysis stage. These observations was probably ascribed to high particulate matter in raw primary sludge, which are hardly transported into bacteria cells for further degradation, lengthening CH_4 production [56]. After getting the peaks, CH_4 production of co-digestion bottles appeared to significantly slow down over time. Despite exhibiting the highest CH_4 production at the first day (340 $mLCH_4$/d), bottles introduced with 1.0% glycerol essentially ceased forming CH_4 in short digestion time, day 4 and around day 6 for 15 mM and 30 mM $NaHCO_3$ added bottles, respectively. As discussed previously, there was a clear relation of system stability and CH_4 production. In the digestate of R-30-1.0 bottles, unfavourable conditions for methanogens, including a drop of pH (from 6.9 to 4.9) and a destruction of buffer capacity (from 2490 to 1758 $mgCaCO_3$/L) could elucidate the short methanogenic activity. Correspondingly, these bottles showed the lowest VS removal efficiency (21%) although they were fed with the highest glycerol (1.0%) and buffer concentrations (30 mM $NaHCO_3$). Similar tendency in terms of pH drop, alkalinity decrease, and inadequate VS removal was observed with R-15-1.0 bottles (Table 19). Meanwhile, bottles treating lower glycerol concentrations had the gradual trend in generating CH_4 and maintained digestion process until around day 14. There would be hence a shock loading for a sustainable anaerobic metabolism with higher dosage of glycerol (1.0%).

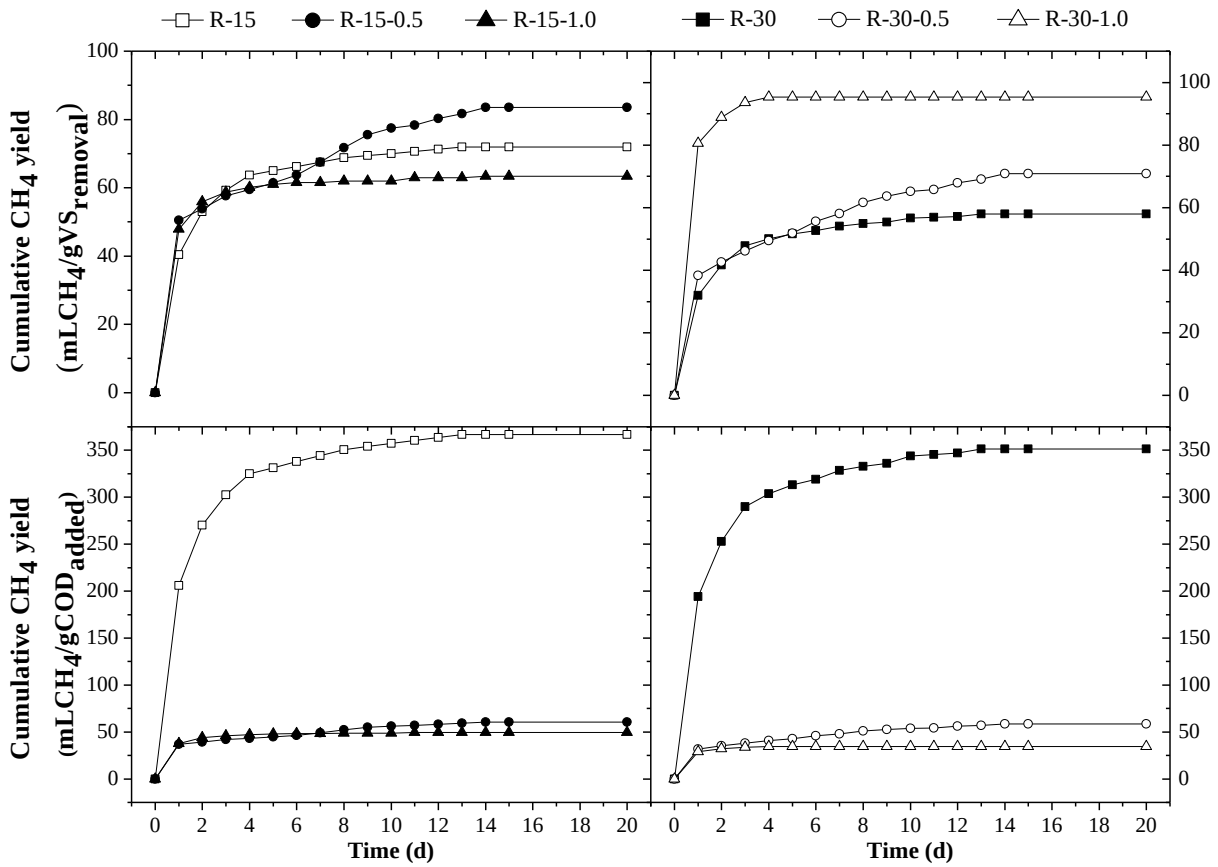


Figure 27. Profile of daily and cumulative CH_4 production in the co-digestion batch test; R-15-0.5, R-15-1.0, R-30-0.5, R-30-1.0 refer to the mixture of 10% digested sludge and 90% raw primary sludge by volume (R-0) supplemented with 15 (R-15) and 30 mM $NaHCO_3$ (R-30), and introduced with 0.5% and 1.0% glycerol each, respectively.

When glycerol content was augmented in co-digestion bottles from 0.5 – 1.0% (v/v) of raw primary sludge, CH_4 production on basis of COD fed was found significantly higher in reference bottles compared to bottles containing glycerol. Irrespective of buffer concentrations added, R-15 and R-30 bottles had around six and seven times higher than corresponding bottles with 0.5% and 1.0% glycerol regarding cumulative CH_4 yields (Figure 28). In spite of an enhanced rapidly biodegradable organic content in the feed, an incomplete conversion of substrate occurred in co-digestion bottles, particularly in the methanogenesis. Insufficient buffer capacity for temporary VFA accumulation was probably one explanation for this system failure. It was even clear with a considerable increase of COD_s in their digestates, which was mainly contributed by VFAs generated from all bottles, especially glycerol-supplied bottles (Table 19). The condition was not suitable for methanogenic activity due to the low I/S ratio as reported normally less than 2 [144, 163]. The early termination of CH_4 production and inadequate CH_4 yields were a consequence of only 10% by volume of digested sludge used in this investigation.

On the other hand, higher CH_4 volume produced as one gram of VS degraded was accomplished in bottles after the addition of glycerol in the feed throughout the whole experimental time. When 30 mM $NaHCO_3$was introduced into BMP bottles, CH_4 yields were 57.9, 70.8, and 95.3 $mLCH_4/gVS_{removal}$ from BMP bottles with 0%, 0.5%, and 1.0% addition of glycerol, respectively (Figure 28). Given the fact that the CH_4 production was proportional to the substrate destruction in a given anaerobic reactor, data given here suggested that co-digesting raw primary sludge with glycerol improved the anaerobic biodegradation of solid matter into CH_4. Nonetheless, it was again noted that the CH_4 yields and VS removal efficiencies were low compared to what have been previously reported (Table 6). This observation could explain the system instability of all co-digestion and reference bottles as previously discussed (Section 5.2).

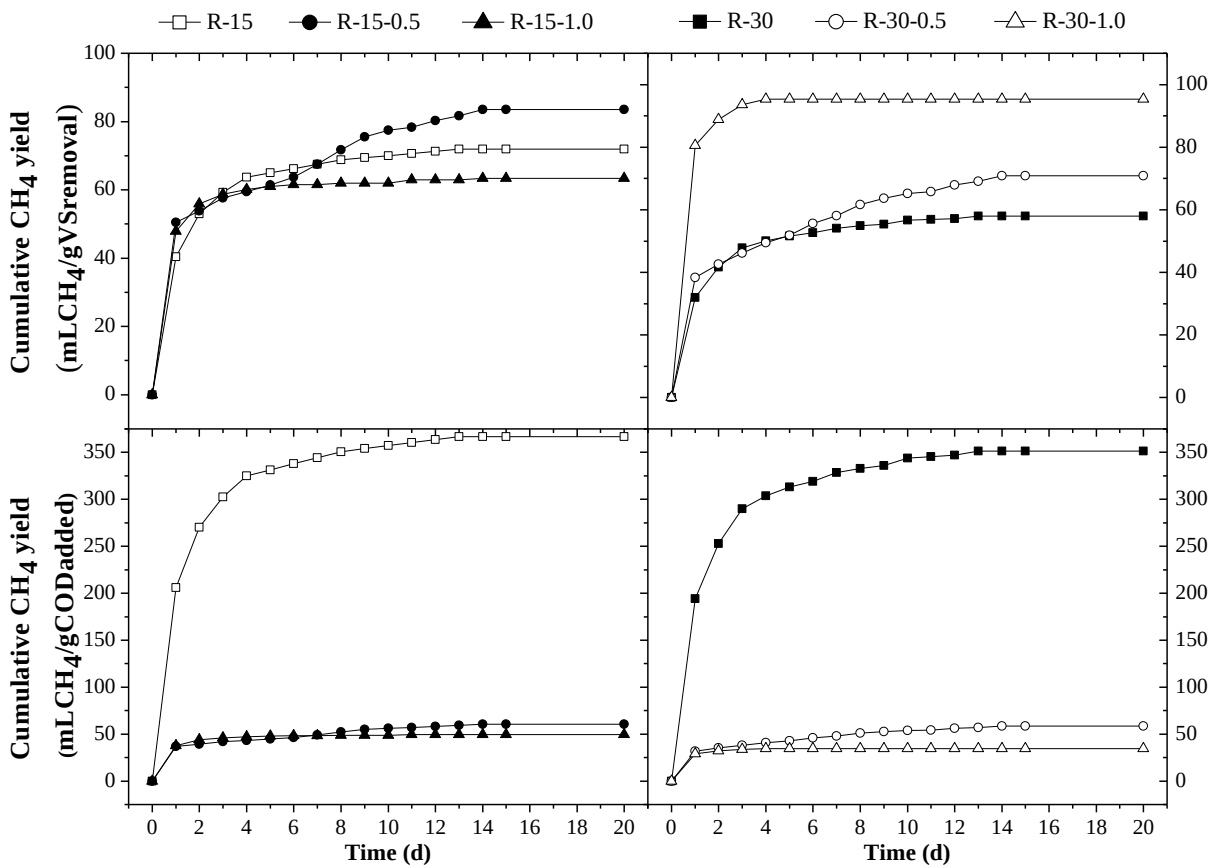


Figure 28. Profile of cumulative CH_4 production on basis of VS removal ($mLCH_4/gVS_{removal}$) and COD added ($mLCH_4/gCOD_{added}$); R-15-0.5, R-15-1.0, R-30-0.5, R-30-1.0 refer to the mixture of digested sludge and raw primary sludge (1/9 by volume) supplemented with 15 (R-15) and 30 mM $NaHCO_3$ (R-30), and introduced with 0.5% and 1.0% glycerol each, respectively.

The first order and modified Gompertz models were tested for cumulative CH_4 production in terms of COD added for anticipating further long-term effects of using glycerol as a co-substrate of raw primary sludge (Figure 29 and Figure 30). Table 20 summaries regression parameters from the first order and modified Gompertz models for the CH_4 production in all reference and co-digestion bottles. Estimated results revealed that both kinetic models successfully monitor CH_4 yields in all cases with high correlation factors (>98%) except for bottles supplied with 15 mM $NaHCO_3$ and 0.5% glycerol with lower correlation coefficients (84 – 90%).

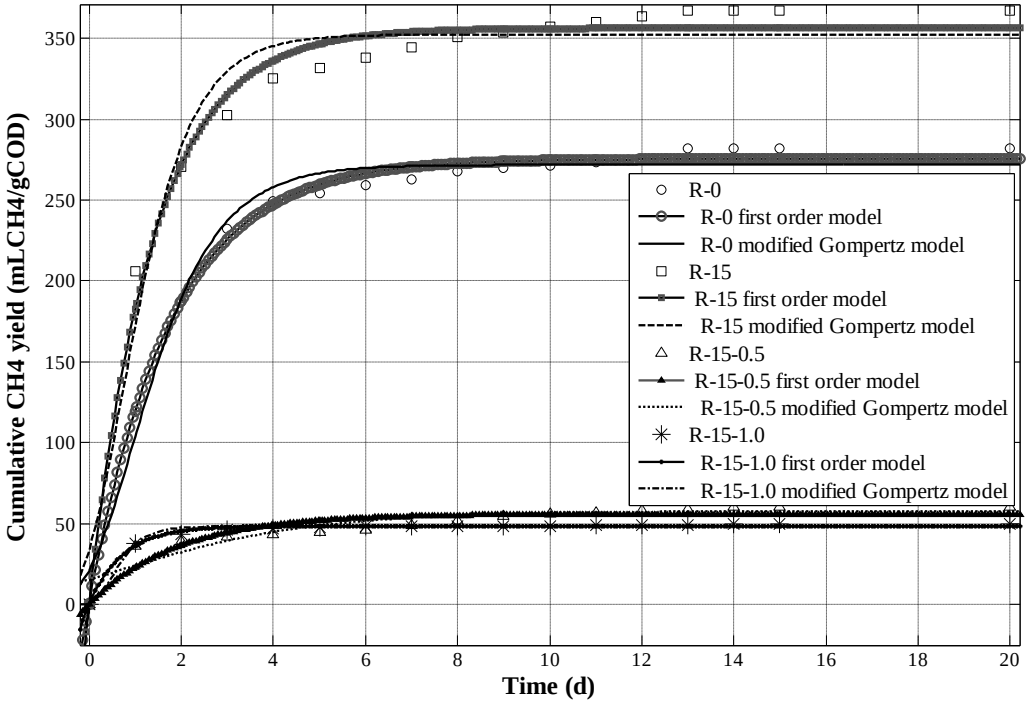


Figure 29. Plots of cumulative CH_4 yields on COD basis and regression fitting curves of the first order and modified Gompertz models; R-15-0.5, R-15-1.0 refer to the mixture of 1/9 I/S supplemented with 15 mM $NaHCO_3$ (R-15) and introduced with 0.5% and 1.0% glycerol respectively.

The first order rate constants (k) were computed at higher values for bottles treating 1.0% glycerol compared to reference bottles; k values were 1.37 and 0.72 1/d in bottles R-15-1.0 and R-15 respectively, for example (Table 20). This indicated that the glycerol addition significantly activated the CH_4 generation. In fact, an instable situation was created in all BMP bottles with early cease of producing biogas, the anaerobic microbes firstly utilised biodegradable matter. This assumption could clearly elucidate the high soundness of the first order model.

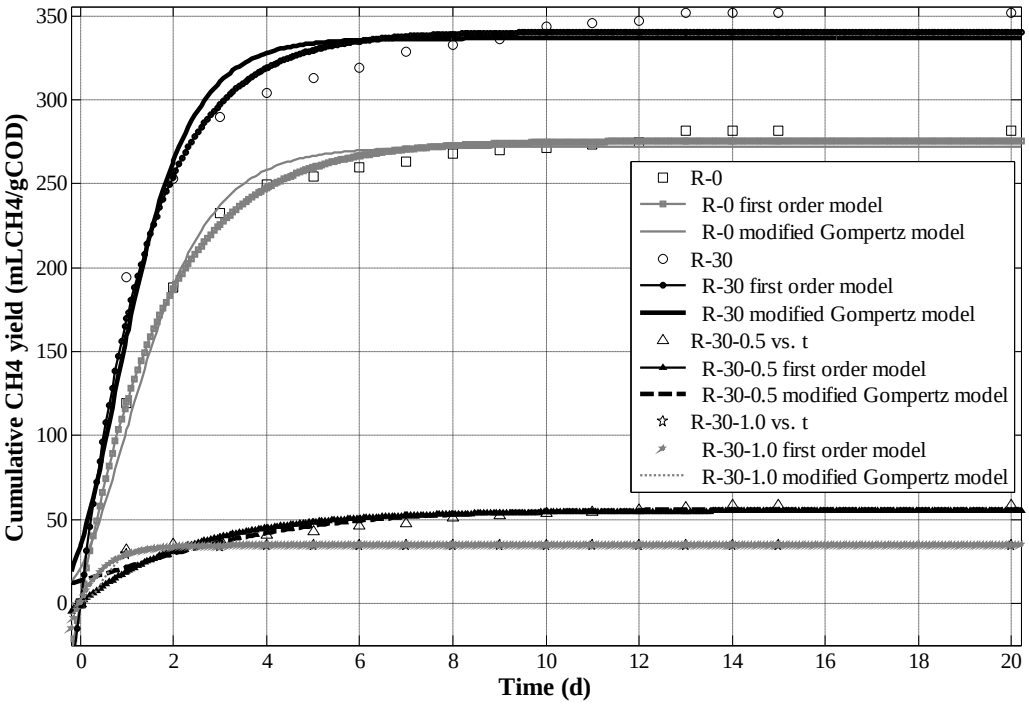


Figure 30. Plots of cumulative CH_4 yields on COD basis and regression fitting curves of the first order and modified Gompertz models; R-30-0.5, R-30-1.0 refer to the mixture of digested sludge and raw primary sludge (1/9 by volume) supplemented with 30 mM $NaHCO_3$ (R-30) and introduced with 0.5% and 1.0% glycerol respectively.

In order to more properly evaluate the trend of CH_4 formation over experimental period, the modified Gompertz model was tested. From this model, two other important parameters, including CH_4 production rate and lag phase time, were obtained. The lag phase time was clearly seen from 1.0 % glycerol-added bottles, reported at 0.14 and 0.17 days with 15 and 30 mM $NaHCO_3$, respectively; while the other bottles immediately produced CH_4. In co-digestion bottles, ultimate CH_4 yields decreased along with increased glycerol addition (0.5 and 1.0%). By contrast, the maximum CH_4 production rates seemed to be achieved higher with more glycerol fed. CH_4 production was therefore simulated but not well maintained over digestion time. It is clear that the influence of glycerol addition on the methanogenesis with respect to yields and stability. In a comparison with reference bottles, higher rates of forming CH_4 were obtained from R-15 and R-30 bottles; however, lower CH_4 production rates were found in these buffered bottles with glycerol addition (Table 20). This is because the anaerobic conversion of glycerol led to rapid VFA

accumulation, declining buffer capacities of co-digestion bottles. The methanogenesis was quickly suspended as a result. Therefore, necessary amount of buffer should be higher for better methanogenic performance in co-digestion context.

Table 20. Kinetic analysis of CH_4 production on COD basis in the batch test of co-digestion of raw primary sludge and glycerol.

Models and kinetic parameters (at 95% confidential intervals)	**R-0**	**R-15**	**R-15-0.5**	**R-15-1.0**	**R-30**	**R-30-0.5**	**R-30-1.0**
Experimental cumulative CH_4 yield ($mLCH_4/gCOD$)	281.7	366.8	60.7	49.7	351.3	58.5	34.5
First order kinetic model							
First order rate constant – k (1/d)	0.570	0.719	0.528	1.367	0.689	0.422	1.793
R^2	0.996	0.986	0.865	0.993	0.983	0.907	0.998
Predicted cumulative CH_4 yield – G_o ($mLCH_4/gCOD$)	275.5 ± 3.4	356.3 ± 7.1	55.9 ± 4.0	48.6 ± 0.6	340 ± 7.7	55.0 ± 0.5	34.4 ± 0.2
Modified Gompertz model							
Lag phase – λ (d)	0.000	0.000	0.000	0.138	0.000	0.000	0.168
R^2	0.983	0.961	0.840	0.988	0.956	0.888	0.996
Maximum CH_4 production rate – R_m ($mLCH_4/gCOD.d$)	97.5 ± 20.4	153.9 ± 49.3	8.8 ± 4.8	47.4 ± 15.3	138.4 ± 46.0	8.2 ± 3.6	42.2 ± 11.4
Predicted cumulative CH_4 yield – G_o ($mLCH_4/gCOD$)	271.8 ± 6.6	352.3 ± 11.7	57.8 ± 5.4	48.4 ± 0.8	336.5 ± 12.0	56.1 ± 4.7	34.4 ± 0.3

R-15-0.5, R-15-1.0, R-30-0.5, R-30-1.0 refer to the mixture of 1/9 I/S supplemented with 15 (R-15) and 30 mM $NaHCO_3$ (R-30), and introduced with 0.5% and 1.0% glycerol each, respectively

For the better understanding of the whole AD process in this investigation, SMA should be taken into account. SMA was a useful tool to evaluate the anaerobic population capacity to convert substrate into CH_4 in given conditions [134, 137, 151, 164]. In the early stage, the buffer expressed its role in simulating bacteria activity; with the higher SMA values were achieved in bottles buffered with $NaHCO_3$ than bottles without buffer (R-0) (Figure 31). BMP bottles with 30 mM $NaHCO_3$ achieved the highest SMA values of 0.91 and 0.96 gCOD/gVS.d, meanwhile the methanogenic activity of 15 mM $NaHCO_3$-added bottles was calculated at 0.75 and 0.72 gCOD/gVS.d for 0.5% and 1.0% treatments, respectively. There was an

independence between glycerol dosage and methanogenic activity, glycerol accordingly caused no inhibitory effects on methanogenic consortium.

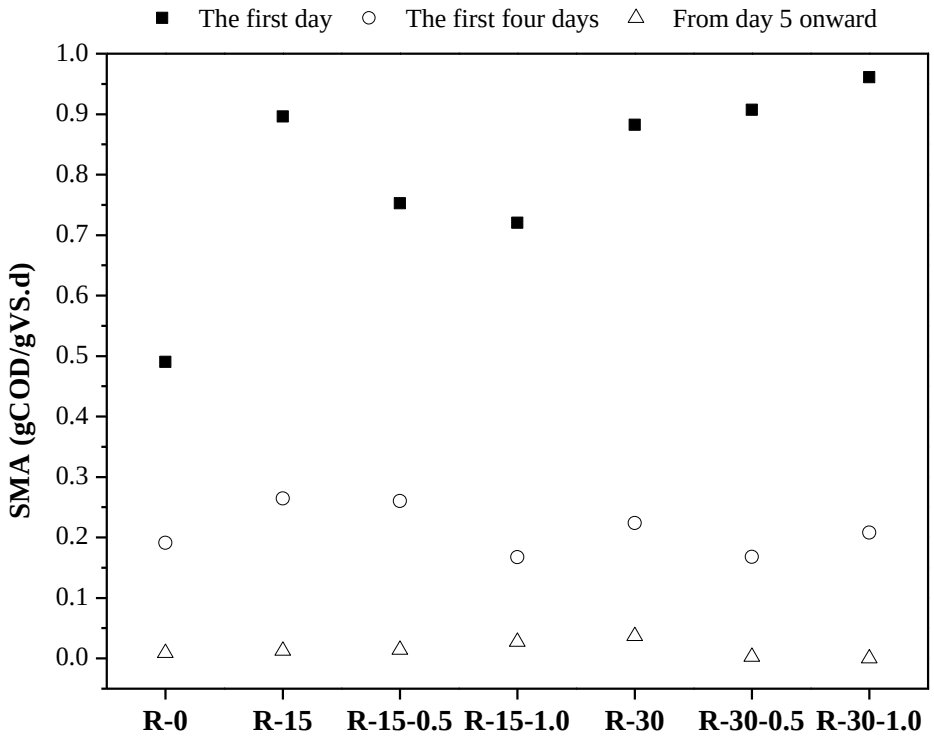


Figure 31. Profile of SMA of reference bottles and co-digestion bottles; R-15-0.5, R-15-1.0, R-30-0.5, R-30-1.0 refer to the mixture of 1/9 I/S supplemented with 15 (R-15) and 30 mM $NaHCO_3$ (R-30), and introduced with 0.5% and 1.0% glycerol each, respectively.

It has been reported that higher SMA values could result in higher the rates of CH_4 generation on VS basis [137]. There was a clear correspondence between of SMA and CH_4 production rate as far as their patterns over the experimental time were concerned. The maximum SMA values were at the first day for all co-digestion and reference bottles, followed by a gradual decrease in bottles without glycerol but an immediate drop in glycerol-contained feeds. Furthermore, when the SMA values in this investigation were averaged for the first five digestion days, healthier biomass activity was observed in bottles containing no glycerol (R-0, R-15, and R-30). From day 5 onwards, on average, all BMP bottles remained low methanogenic activity; even a suspension of CH_4-forming bacteria was found in R-30-1.0 bottles (Figure 31). In the long-term operation, it can be concluded that this reduction in SMA was associated to excess organic matter and the insufficient bacterial function, expressed

as the low I/S ratio of 1/9 by volume tested. This finding was confirmed by Souto et al. [134] who found that SMA values were more dependent on the ratio of food and microorganism than substrate concentration only. Moreover, considering SMA greatly depends on the particular operational conditions and digestion duration [134, 145], it is evitable to further investigate various ratios of inoculum amount and glycerol concentrations under definite conditions to determine the threshold for a stable AD process.

In summary, the supplement of 0.5% and 1.0% of glycerol into raw primary sludge was satisfactory in terms of CH_4 production within the start-up stage. This improvement resulted from the enhancement of organic source with regard to COD_s, the highly biodegradability and solubility of glycerol. Nonetheless, certain characteristics of digestates, typical of higher COD_s, acidic pH and inadequate alkalinity as well as short methanogenic duration, indicated the incomplete anaerobic biodegradation for the prolonged operation. Therefore, in terms of system stability, additional carbon matter from glycerol only proved its positive effects on the hydrolytic-acidogenetic stage rather than methanogenesis. These results indicated that glycerol could be used as a co-substrate; however, the appropriate I/S ratio and buffer supplementation should be further studied in order to prevent overloading and attain the ultimate CH_4 potential.

5.4 Summary

Data from this anaerobic co-digestion test of raw primary sludge and glycerol pointed out that the addition of 0.5% and 1.0% of glycerol into raw primary sludge was satisfactory in terms of daily and cumulative CH_4 production within the start-up stage. This improvement resulted from the enhancement of organic source with regard to COD_s, the highly biodegradability and solubility of glycerol. On the other hand, certain characteristics of digestates, namely higher COD_s, acidic pH and inadequate alkalinity as well as short methanogenic duration, indicated the incomplete anaerobic biodegradation for the long-term operation. These findings indicated effects of I/S ratio and buffer supplementation on system stability and then the ultimate CH_4 potential. Their appropriate levels should be further investigated to optimize CH_4 generation of anaerobic co-digestion.

6 FATE OF TRACE ORGANIC COMPOUNDS DURING ANAEROBIC DIGESTION OF SEWAGE SLUDGE

This chapter aims to evaluate the removal efficiency of TrOCs under anaerobic condition and their biodegradation as well as distribution between the aqueous (water) and solid (sludge) phases. The overall performance of the AD process was examined with respect to biogas production and COD removal, followed by the removal of these TrOCs and their behaviour in the water and solid phases.

6.1 Overall performance of AD of trace organics

The overall performance of the anaerobic degradation of trace organic-spiked sludge was evaluated by focusing on the evolution of the variation of relevant parameters, namely pH, alkalinity, ammonia nitrogen, organic acids, VS/TS ratio, VS and COD_s, and the production of CH_4 throughout the digestion time.

6.1.1 The variation of control parameters during over the digestion time

Table 21 presents key characteristics of digested sludge and digested sludge spiked with TrOCs. Data from BMP experiments using only digested sludge were used as the reference. This table also shows the variations of these characteristics in TrOC-spiked bottles at a particular time during the digestion time.

After spiking, no considerable change in most parameters was observed. In particular, pH values were 7.8 for reference and TrOC-spiked bottles; accordingly, alkalinity values in theses bottles were similar (4233 and 4316 mg$CaCO_3$/L for digested sludge without and with TrOCs, respectively). TS and VS content were slightly higher in reference bottles compared to spiked bottles (27.0 gTS/L and 17.5 gVS/L in bottles before spiking and 25.8 gTS/L and 16.3 gVS/L after spiking, respectively). However, the VS/TS ratio remained unchanged and was 64% in both cases. COD_s increased significantly with the addition of TrOCs, due to the addition of the stock solution of TrOCs. The amount of TrOCs (135 μg/L) used here did not cause any change in COD_s concentration. The increase in COD_s concentration is from methanol, which was used as the solvent for the preparation of TrOC stock solution.

Table 21. Characteristics of digested sludge bottles and TrOC-spiked bottles throughout the experimental time.

Characteristics		Sample mixtures								
		DS	**R-0**	**R-2**	**R-6**	**R-9**	**R-14**	**R-21**	**R-28**	**R-35**
Content	Anaerobic digested sludge (mL)	750	750	750	750	750	750	750	750	750
	TrOCs (μg/L)	nd	135	135	135	135	135	135	135	135
	Day of sampling (n^{th} day)	nd	0	2	6	9	14	21	28	35
Parameters	TS (g/L)	27.0	25.8	25.6	26.4	24.3	24.8	24.1	23.7	24.2
	VS (g/L)	17.5	16.3	15.9	16.5	15.3	15.0	14.6	14.1	14.5
	VS/TS (%)	64.9	63.3	62.1	62.2	62.9	60.4	60.5	59.7	59.9
	pH	7.8	7.9	7.6	7.7	7.8	7.8	7.8	8.3	7.8
	Alkalinity ($mgCaCO_3/L$)	4233	4316	4211	4389	4237	4115	4665	2374	2656
	COD_t (g/L)	28.4	26.0	29.6	28.2	21.3	23.1	20.7	21.7	19.5
	COD_s (g/L)	0.44	3.51	3.61	1.67	0.89	0.95	1.07	0.76	1.08
	COD_s/COD_t (%)	1.5	13.5	12.2	5.9	4.2	4.1	5.2	3.5	5.5

Digested sludge (DS); nd = no data; R-0, R-2, R-6, R-9, R-14, R-21, R-28, and R-35 refer to TrOC-spiked digested sludge bottles sampled at day 0, 2, 6, 9, 14, 21, 28, and 35, respectively; All data are expressed as mean values

Throughout the experiment, values of chemical control parameters as a function of time were in the desirable range for the AD process. The pH varied in the range of 7.6 – 8.3, which was favourable for the normal growth of anaerobic microbes. The stable values of pH can be attributed the relatively high buffer capacities of TrOC-spiked bottles with an initial alkalinity values of 4316 $mgCaCO_3/L$. Figure 32 presents the variation of pH and alkalinity during the course of digestion. Alkalinity values remained stable at the value of more than 4000 $mgCaCO_3/L$ until day 21 and reduced to more than 2000 $mgCaCO_3/L$ for the remaining period of digestion. These values are compatible with those previously reported for the normal growth of anaerobic microbes [44].

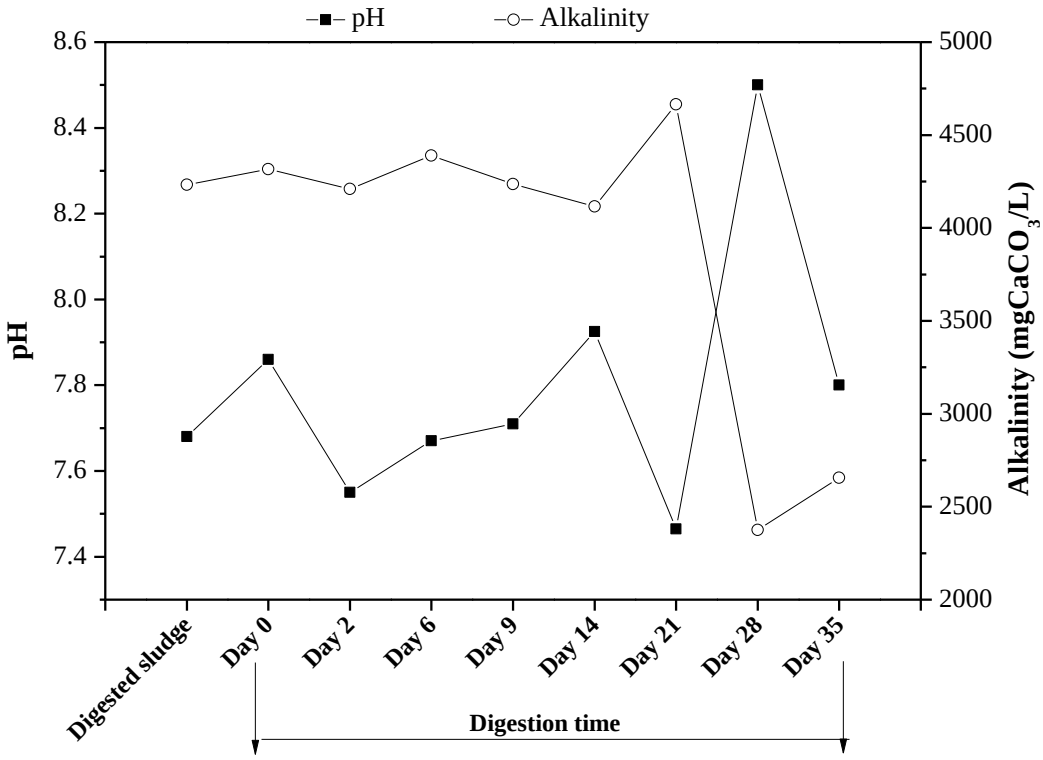


Figure 32. Profile of pH and alkalinity values in digested sludge bottles and spiked digested sludge bottles at the particular digestion time.

Because digested sludge was used as the inoculum, the reductions in TS and VS over 35 days were small (Table 21). However, the removal of soluble organic matter measured by COD_s was significant (Figure 33). The solubilisation and acid formation of methanol in the hydrolytic-acidogenic stage were responsible for such increase. From day 2, a significant decrease in COD_s could be observed reaching a stable concentration at around $1gCOD_s/L$ after day 9. Due to an insignificant variation of COD_t over the digestion time, it is not surprising that the ratio of COD_s and COD_t exhibited the same pattern with COD_s during the course of incubation.

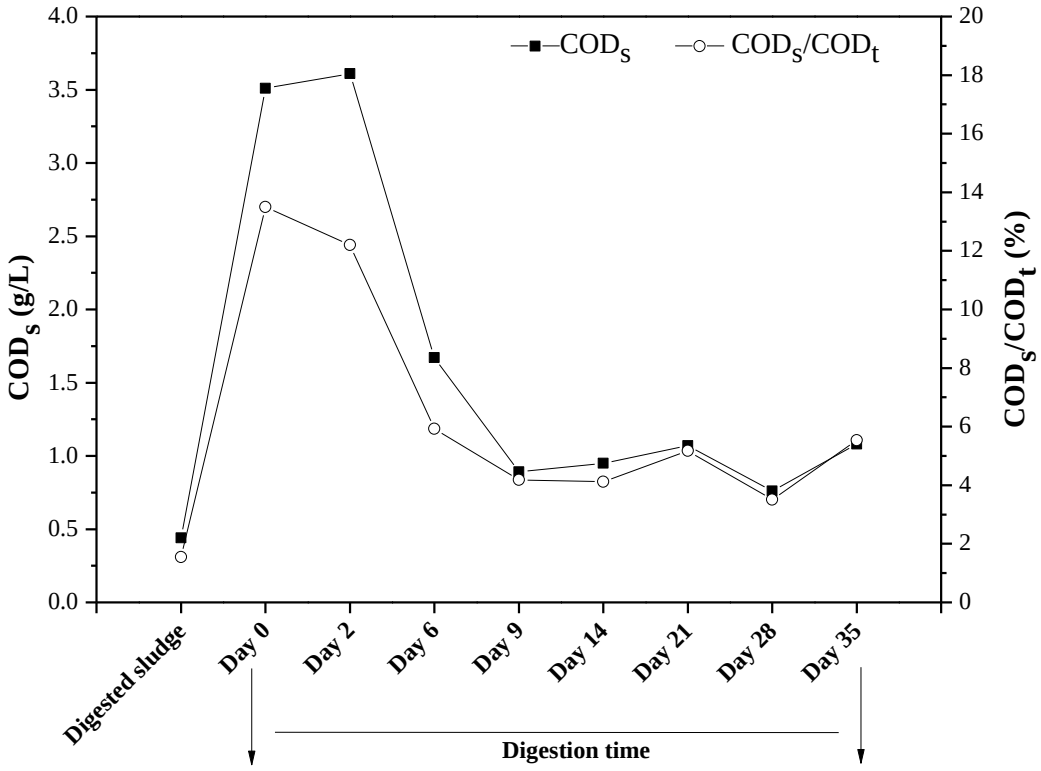


Figure 33. Profile of COD_s and ratio of COD_s/COD_t of digested sludge and digested sludge spiked with TrOCs from ay 0 to day 35 of digestion.

6.1.2 Anaerobic conversion of trace organic compounds

Figure 34 shows the daily and cumulative CH_4 produced from tested bottles versus those from reference bottles. Throughout the experiment, the similar performance of methanogenesis in terms of daily production was observed in both reference and spiked bottles. The only exception is that CH_4 production from bottles containing TrOCs was significantly higher to that of the reference bottles from day 4 to 9. This initial high CH_4 yield in TrOCs-spiked bottles could be attributed to the addition of methanol, which was used as the solvent in preparing the TrOC stock solution. Methanol is a readily amenable carbon source. After the biodegradation of methanol in day 5 to day 8, which resulted in a high CH_4 production rate, the process returned to normal, and CH_4 productions from the TrOC spiked and unspiked bottles were similar. These results confirm that the addition of TrOCs in methanol did not cause any inhibitory effects to the AD process.

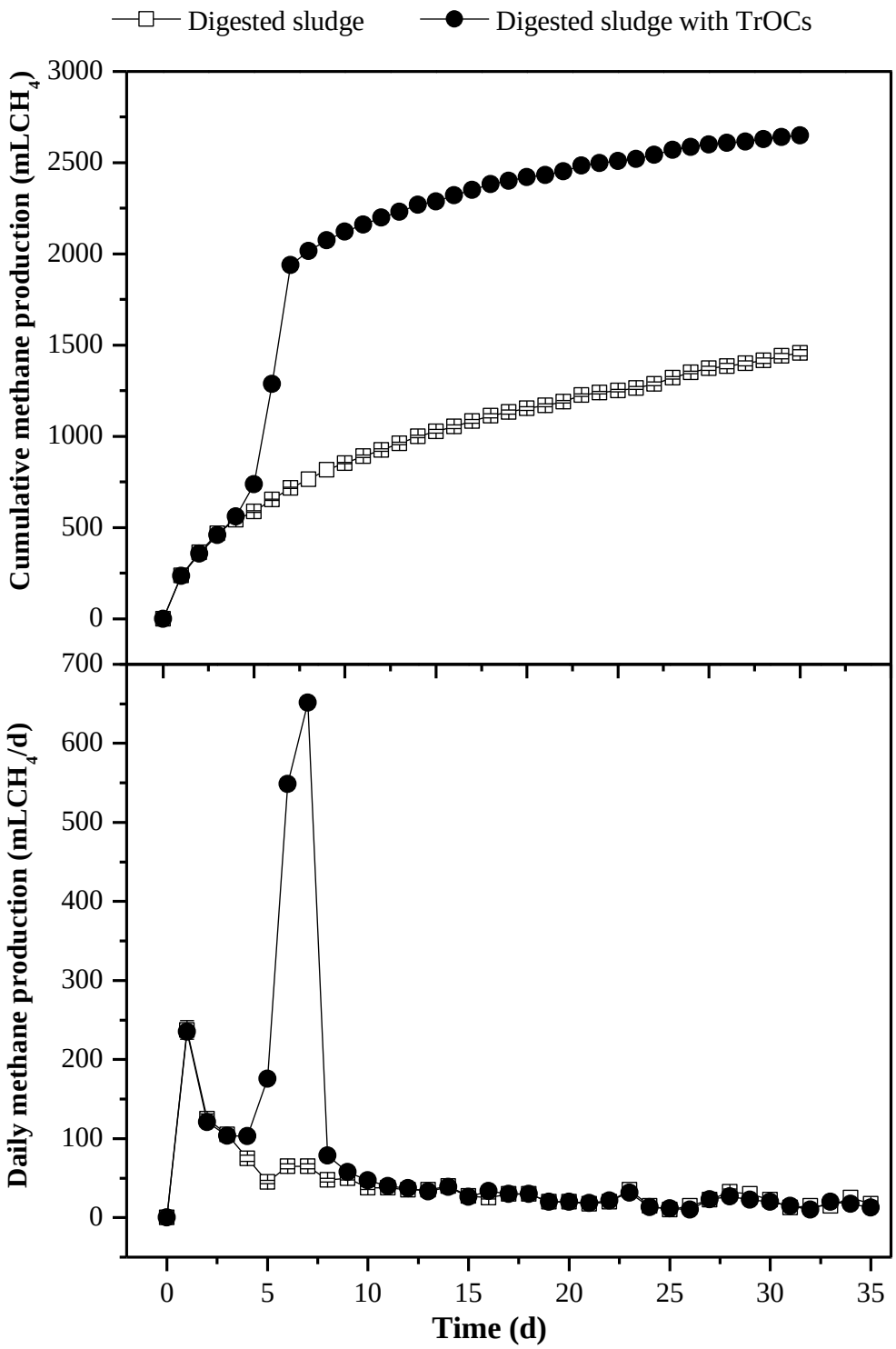


Figure 34. Profile of daily and cumulative CH_4 production of reference and TrOC-spiked MBP bottles over 35 days; error bars refer to the standard deviation (n = 2).

The first order and modified Gomperzt kinetic models were used to describe the CH_4 yields as a function of time (Figure 35). The first order kinetic model could not

closely match the trend of CH_4 yields from TrOC-spiked bottles (that contained methanol) but could successfully describe the CH_4 yields of the reference bottles. This is possibly due to the fact that the microbial consortium in digested sludge used in this batch test was not previously acclimatised with methanol, resulting in a lag time of CH_4 production. The inclusion of lag time parameter in the modified Gompertz allows it to describe very well the CH_4 yields in all cases in this study (Table 22).

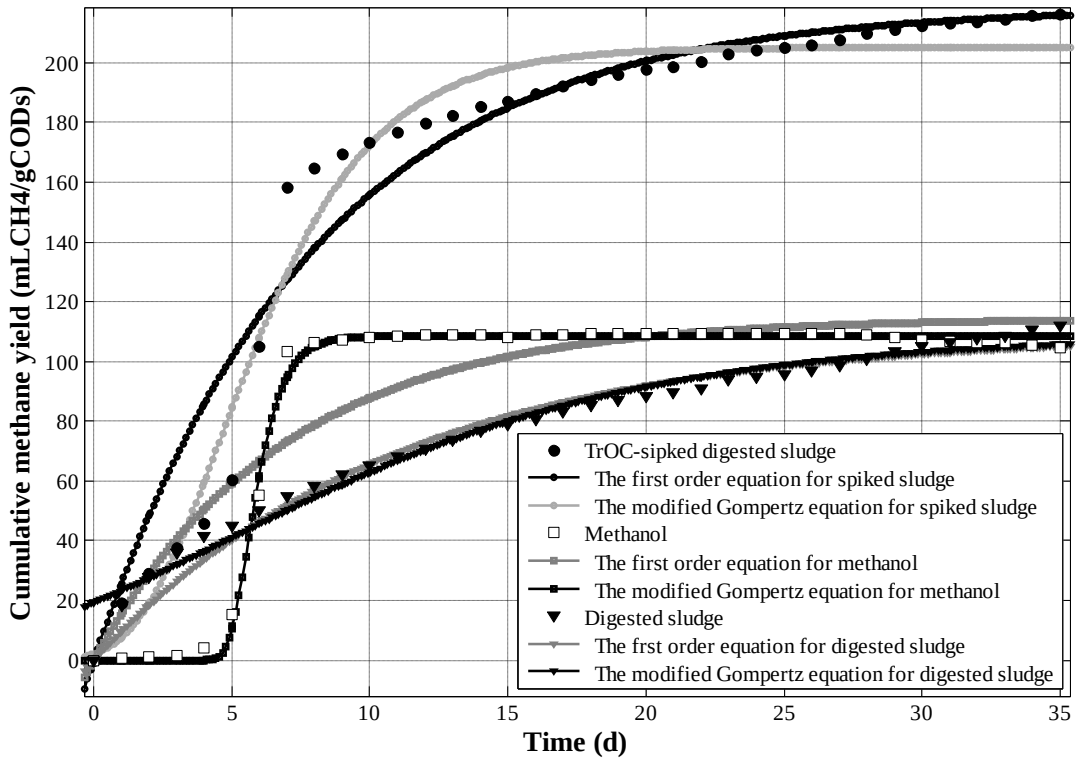


Figure 35. Plots of cumulative CH_4 yields on VS basis and regression fitting curve using the first order and modified Gompertz models applied for digested sludge, TrOC-spiked digested sludge, and methanol.

Based on the modified Gompertz equation, the lag time were 0, 1.6, and 4.9 days for digested sludge, TrOC-spiked digested sludge, and methanol, respectively. In comparison, the longer lag time for the methanogenesis of methanol suggested the inhibitory effects of methanol on anaerobic microbes although methanol has been known as a readily degradable substrate for methanogens [165]. The CH_4 production from bottles treating TrOCs was only suspended for around the first 1.6 days. Meanwhile, it is clear that the rapid CH_4 generation was found in reference bottles, in which digested sludge already reached its stable performance. Considering the

process performance in terms of parameter variations and CH_4 yields, a steady state of methanogenesis was created in TrOC-spiked bottles from day 1.6 onward.

Table 22. Kinetic analysis of CH_4 production on VS basis in the batch test of the AD treating trace organic contaminants.

Models and kinetic parameters (at 95% confidential intervals)	TrOC-spiked digested sludge	Digested sludge	Methanol
Experimental cumulative CH_4 yield at day 35 ($mLCH_4/gVS_{added}$)	216.3	112.0	104.4
First order kinetic model			
First order rate constant – k (1/d)	0.125	0.092	0.146
Correlation coefficient – R^2	0.946	0.976	0.803
Predicted cumulative CH_4 yield – G_o ($mLCH_4/gVS_{added}$)	218.6 ± 10.5	109.5 ± 4.4	114.4 ± 10.7
Modified Gompertz model			
Lag phase – λ (d)	1.591	0.000	4.897
Correlation coefficient – R^2	0.974	0.933	0.996
Maximum CH_4 production rate – R_m ($mLCH_4/gVS_{added}.d$)	24.7 ± 3.9	6.8 ± 0.6	57.0 ± 7.8
Predicted cumulative CH_4 yield – G_o ($mLCH_4/gVS_{added}$)	205.1 ± 4.9	101.8 ± 4.4	57.8 ± 5.4

6.2 Dynamics of trace organics under anaerobic sludge treatment

The digested sludge used in this study was obtained from a full-scale plant. Thus, TrOCs were detected in the water and solid phases of digested sludge prior to spiking (Figure 36). However, these background concentrations were small. With the exception of carbamazepine and triclosan, their concentrations in the solid phase were well below 5 µg/g.

In wastewater sludge, TrOCs could adsorb onto the solid phase (sludge) and remain in the water phase [166]. Therefore, both water and solid contributions made up the concentration of TrOCs in sludge samples. Concentrations of TrOCs in all sludge samples are calculated as:

$$C = Cs + Cw \quad (11)$$

$$\text{and } Cs = X \times TS \quad (12)$$

where X (µg/g) and Cs (µg/L) refer to TrOC concentrations in solid phase; Cw (µg/L) are the concentrations of TrOCs in liquid phase, and TS is the solid concentration of samples (g/L).

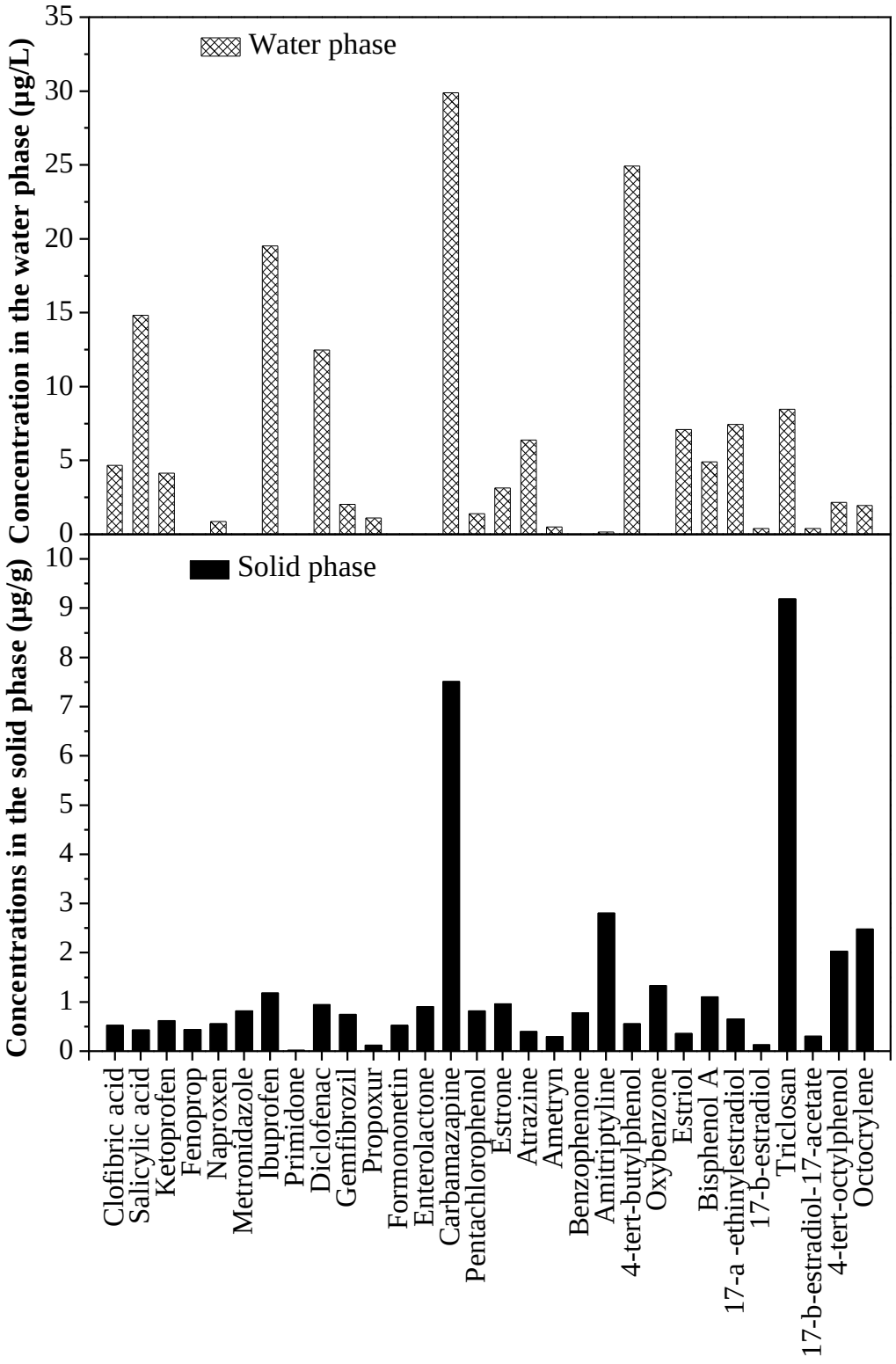


Figure 36. Distribution of the selected TrOCs in the water and solid phases of digested sludge.

During digestion, mass transfer of TrOCs between the aqueous and solid phases as well as biodegradation regulated the evolution of their concentrations. Their mass

transfer into air phase (known as volatilisation) was neglected due to their Henry's law constant of tested compounds were calculated to be lower than 1.0×10^{-3}atm.m^3/mol (Table 26 – Appendix). The abiotic loss by photodegradation of TrOCs in this study was also minimised by covering BMP bottles to avoid light exposure. The mass transfer of TrOCs then virtually occurred between water and solid compartments of sludge. Hence, the fate of these compounds during the AD of sludge, two-compartment matrix, involves their elimination by biodegradation and the mass transfer from the water phase to the solid phase (sorption) and vice versa (desorption). Their dynamic mode could be schematically presented in Figure 37.

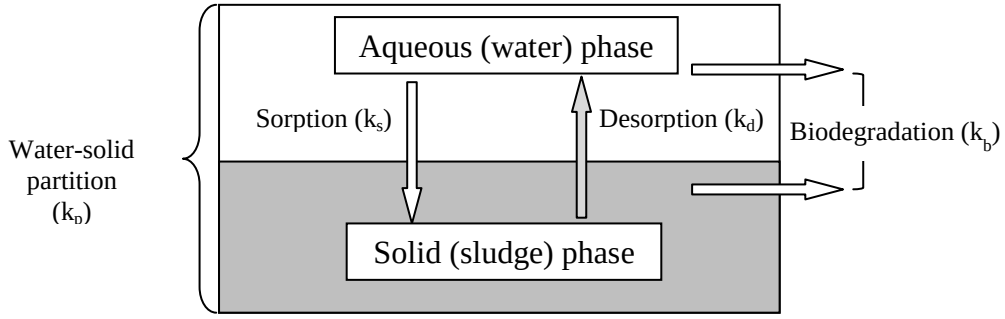


Figure 37. The schematic diagram of the fate of TrOCs in a sludge-water system.

The two-phase fate model can be simplified based on the following assumptions:

- Anaerobic biomass growth is negligible along with the steady operation of all BMP bottles; accordingly, biomass (expressed as VS content) could be considered stable over experimental time; and
- There is no inhibition to methanogenic populations caused by TrOCs as discussed above due to the process stability over experimental time.

As a result, the behaviour of each compound in this study was only examined via anaerobic biodegradation and mass transfer in and between two phases of sludges.

When stability of biomass growth was stable, the pseudo first-order kinetic was preferred to describe biodegradation rate from the water phase only as following [167]:

$$r_b = -k_b \times VS \times C \quad (13)$$

where r_b is the biodegradation rate (μg/L.d), k_b is the biodegradation rate constant in water (L/gVS.d), VS is biomass concentration (gVS/L), C is the initial concentration of TrOC (μg/L).

The transfer rate between the water phase and solid phase followed the linear isotherm. The sorption rate was calculated as:

$$r_s = k_s \times TS \times Cw \quad (14)$$

where r_s is the sorption rate (μg/gTS.d), k_s is the sorption rate constant (L/gTS.d); TS is total solid (gTS/L); and Cw is the initial concentration of TrOC in the water phase(μg/L); meanwhile, desorption rate was:

$$r_d = k_d \times Cs \quad (15)$$

where r_s is the desorption rate (μg/L.d), k_d is the desorption rate constant (1/d); Cs is the initial concentration of TrOC in the solid phase (μg/L).

As an instantaneous equilibrium was created, there was an equal amount of TrOCs transferring between two phases at an infinite time:

$$\begin{aligned} & r_s = r_d \\ & \rightarrow k_s \times TS \times Cw = k_d \times Cs \\ & \rightarrow k_p = \frac{k_s}{k_d} = \frac{Cs}{Cw \times TS} \end{aligned} \quad (16)$$

where k_p is the water-solid partition coefficient (L/g).

The dynamic of each TrOC concentration with time was expressed as a differential equation, in the water phase:

$$\begin{aligned} & \frac{dCw}{dt} = \frac{\Delta C}{\Delta t} = -k_s \times TS \times Cw + k_d \times Cs - k_b \times VS \times C \\ & \rightarrow \frac{dCw}{dt} = -k_s \times TS \times Cw + \frac{k_s}{k_p} \times Cs - k_b \times VS \times C \end{aligned} \quad (17)$$

and in the solid phase:

$$\begin{aligned} & \frac{dCs}{dt} = \frac{\Delta C}{\Delta t} = k_s \times TS \times Cw - k_d \times Cs \\ & \rightarrow \frac{dCs}{dt} = k_s \times TS \times Cw - \frac{k_s}{k_p} \times Cs \end{aligned} \quad (18)$$

The two-phase fate model associated to the dynamic of each TrOC compound in two phases was expressed as a system of differential equations:

$$\begin{cases} \frac{dCw}{dt} = -k_s \times TS \times Cw + \frac{k_s}{k_p} \times Cs - k_b \times VS \times C \\ \frac{dCs}{dt} = k_s \times TS \times Cw - \frac{k_s}{k_p} \times Cs \end{cases} \quad (19)$$

Optimisation of the model parameters in accordance with the experimental data was carried out by using least-squares regression in Matlab.

The total concentration of one specific compound over time was the sum of concentrations in the water and solid phases, expressed as:

$$\frac{d(Cw+Cs)}{dt} = -k_b \times VS \times C \quad (20)$$

The variation of TrOC concentrations over the digestion time followed the pseudo first-order equation:

$$C_t = C_o \times e^{-k_b \times VS \times t} \quad (21)$$

whereC_t and C_o is the initial concentration (t = 0) and the remaining substrate concentration at time t (µg/L), t is the time period (d), and k_b is the biodegradation rate constant (L/gVS.d), VS is biomass concentration (g/L).C_o was the sum of the available concentration in water and solid phases after TrOCs were spiked into samples at the time t = 0:

$$C_o = Cs_o + Cw_o \quad (22)$$

Removal efficiency (R%) at time t was calculated as:

$$R = \frac{C_o - C_t}{C_o} \times 100\% \quad (23)$$

Their half-life time, defined as a time that 50% concentration of TrOCs remained, is calculated as:

$$t_{1/2} = \frac{\ln 2}{-k_b \times VS} \quad (24)$$

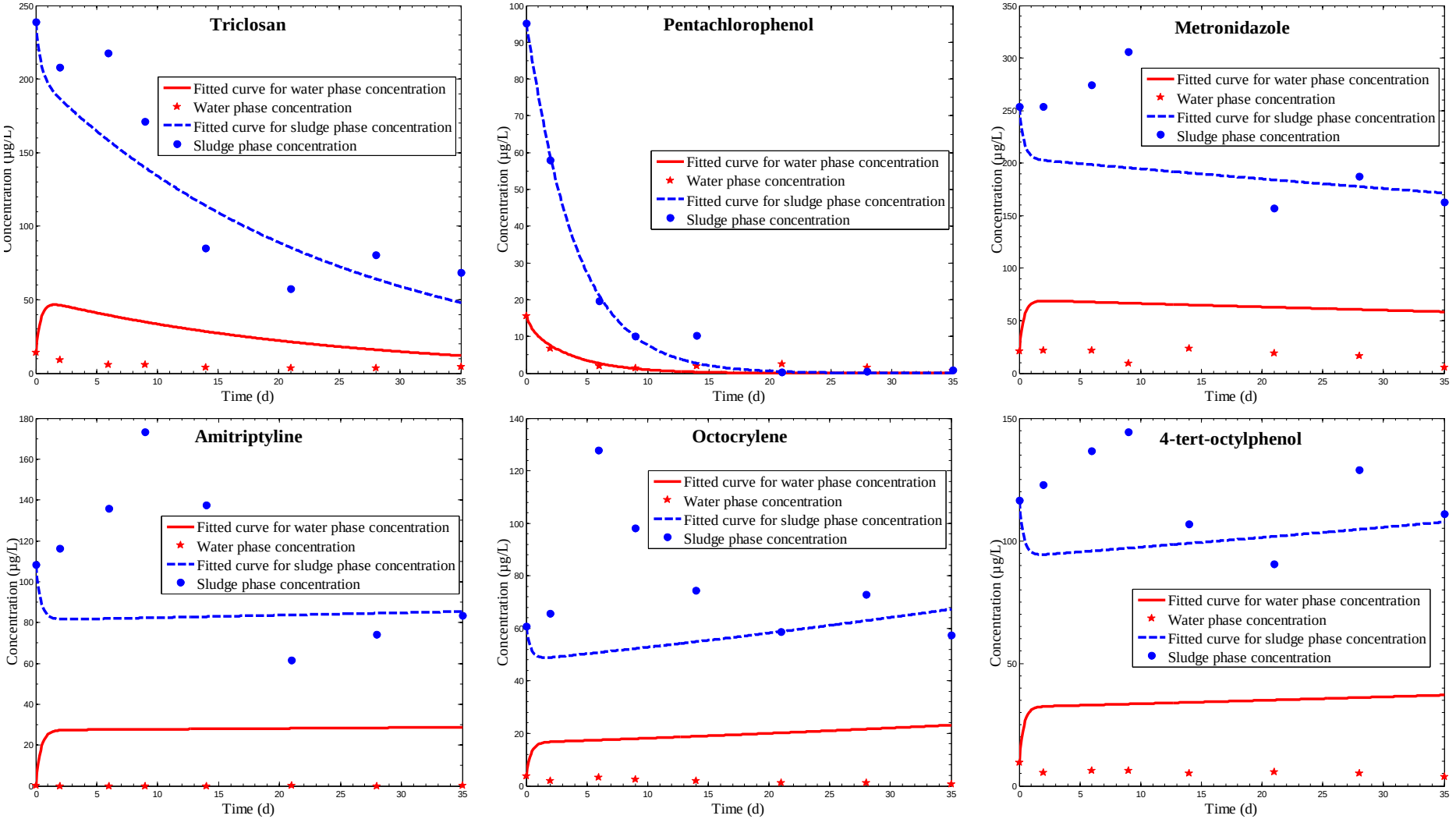
where $t_{1/2}$ is half-life time (d).

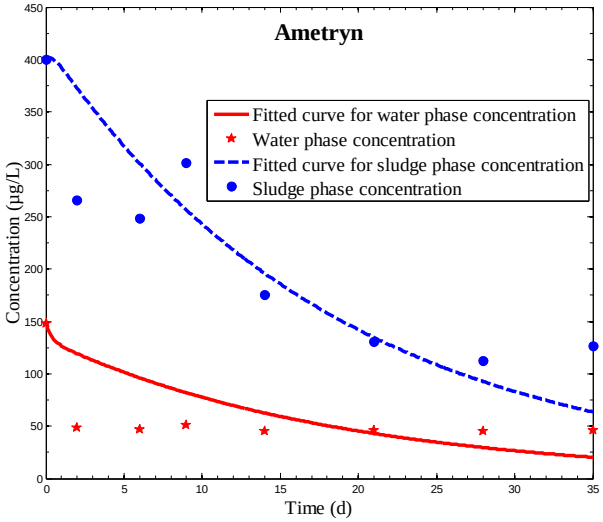
Table 23 summarises value of k_s, k_p, k_b, and $t_{1/2}$ determined from the two phase fate model using experimental data presented in Figure 38.

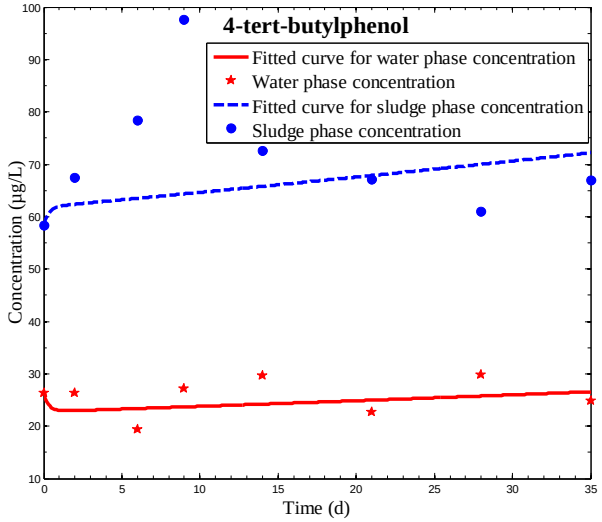
Table 23. Calculated model parameters achieved from the two-phase fate model.

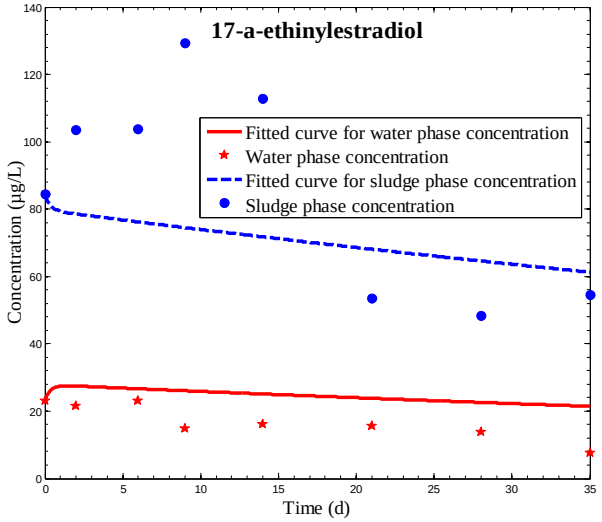
Group	Compound	Log *D* at pH = 8	Model parameters			
			k_s	k_p	k_b	$t_{1/2}$
Pharmaceuticals	Salicylic acid	–1.14	0.169	0.011	0.0229	2.0
	Ketoprofen	–0.55	0.138	0.009	0.0009	51.2
	Naproxen	–0.18	0.175	0.016	0.1243	0.4
	Metronidazole	–0.14	0.080	0.116	0.0003	137.4
	Ibuprofen	0.14	0.131	0.038	0.0000	-
	Primidone	0.83	0.154	0.010	0.0009	50.0
	Diclofenac	1.06	0.091	0.075	0.0042	11.1
	Gemfibrozil	1.18	0.142	0.029	0.0003	162.8
	Carbamazepine	1.89	0.092	0.102	0.0013	36.7
	Amitriptyline	3.21	0.076	0.119	0.0000	-
	Triclosan	4.92	0.078	0.148	0.0027	16.9
Steroid hormones	Estriol	2.53	0.089	0.110	0.0005	84.0
	Estrone	3.62	0.112	0.076	0.0018	25.1
	17-α-ethinylestradiol	4.11	0.086	0.113	0.0005	92.4
	17-β-estradiol	4.15	0.087	0.097	0.0034	13.4
	17-β-estradiol-17-acetate	5.11	0.129	0.056	0.0539	0.9
Pesticides	Clofibric acid	–1.29	0.129	0.039	0.0003	140.8
	Fenoprop	–0.28	0.124	0.021	0.0013	35.8
	Propoxur	1.54	0.120	0.035	0.0026	18.1
	Pentachlorophenol	2.19	0.065	0.140	0.0171	2.7
	Atrazine	2.64	0.113	0.031	0.0078	5.9
	Ametryn	2.97	0.078	0.115	0.0036	12.9
Industrial chemicals	4-tert-butylphenol	3.39	0.089	0.109	0.0000	-
	Bisphenol A	3.64	0.088	0.112	0.0000	-
	4-tert-octylphenol	5.18	0.083	0.117	0.0000	-
Phytoestrogen	Enterolactone	1.88	0.130	0.016	0.0100	4.6
	Formononetin	1.81	0.109	0.052	0.0202	2.3
UV filters	Benzophenone	3.21	0.088	0.112	0.0000	-
	Oxybenzone	3.42	0.156	0.058	0.1797	0.3
	Octocrylene	6.89	0.081	0.118	0.0000	-

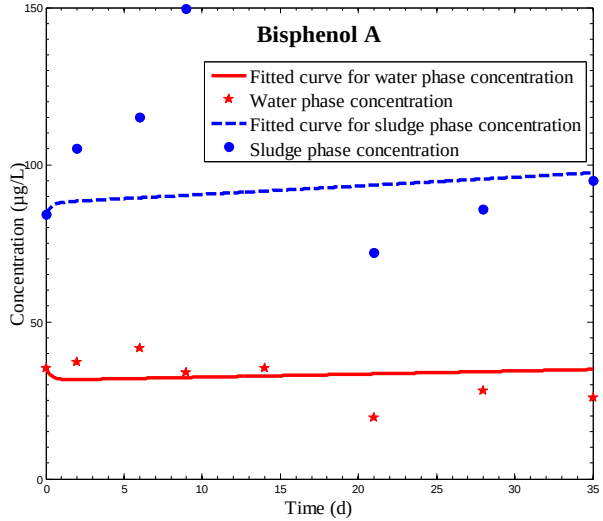
Sorption rate constant (k_s, μg/L.d), water-solid partition coefficient (k_p, L/g), biodegradation rate constant (k_b, L/gVS.d), half-life time ($t_{1/2}$, d); values of log D at pH = 8 were sourced from Scifinder Scholar database (ACD/Labs)

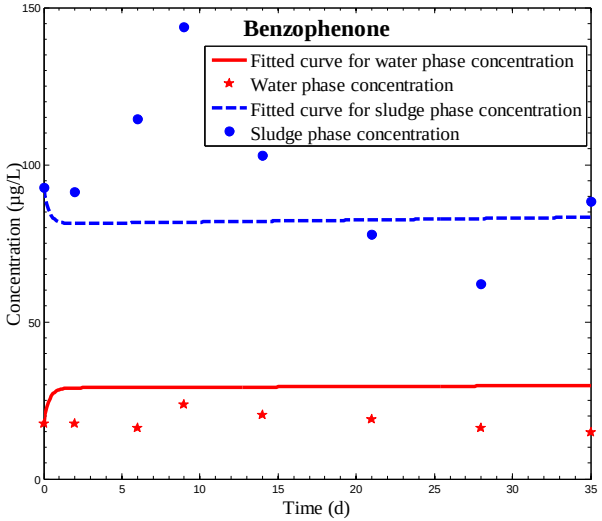

Triclosan
Pentachlorophenol
Metronidazole
Amitriptyline
Octocrylene
4-tert-octylphenol
Fitted curve for water phase concentration
Water phase concentration
Fitted curve for sludge phase concentration
Sludge phase concentration
Concentration (μg/L)
Time (d)

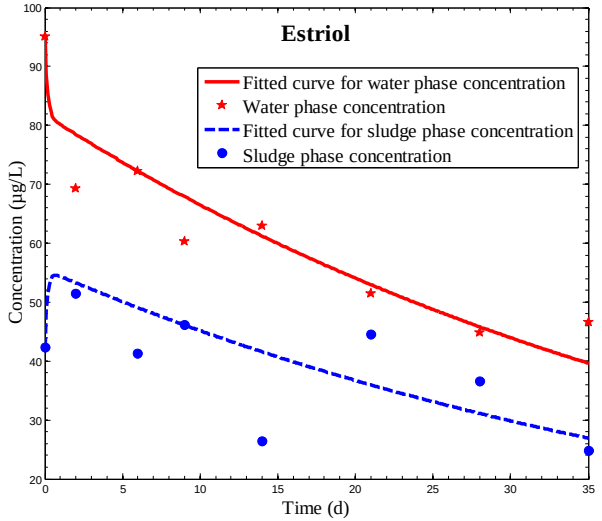

Ametryn
Fitted curve for water phase concentration
Water phase concentration
Fitted curve for sludge phase concentration
Sludge phase concentration
Concentration (μg/L)
Time (d)

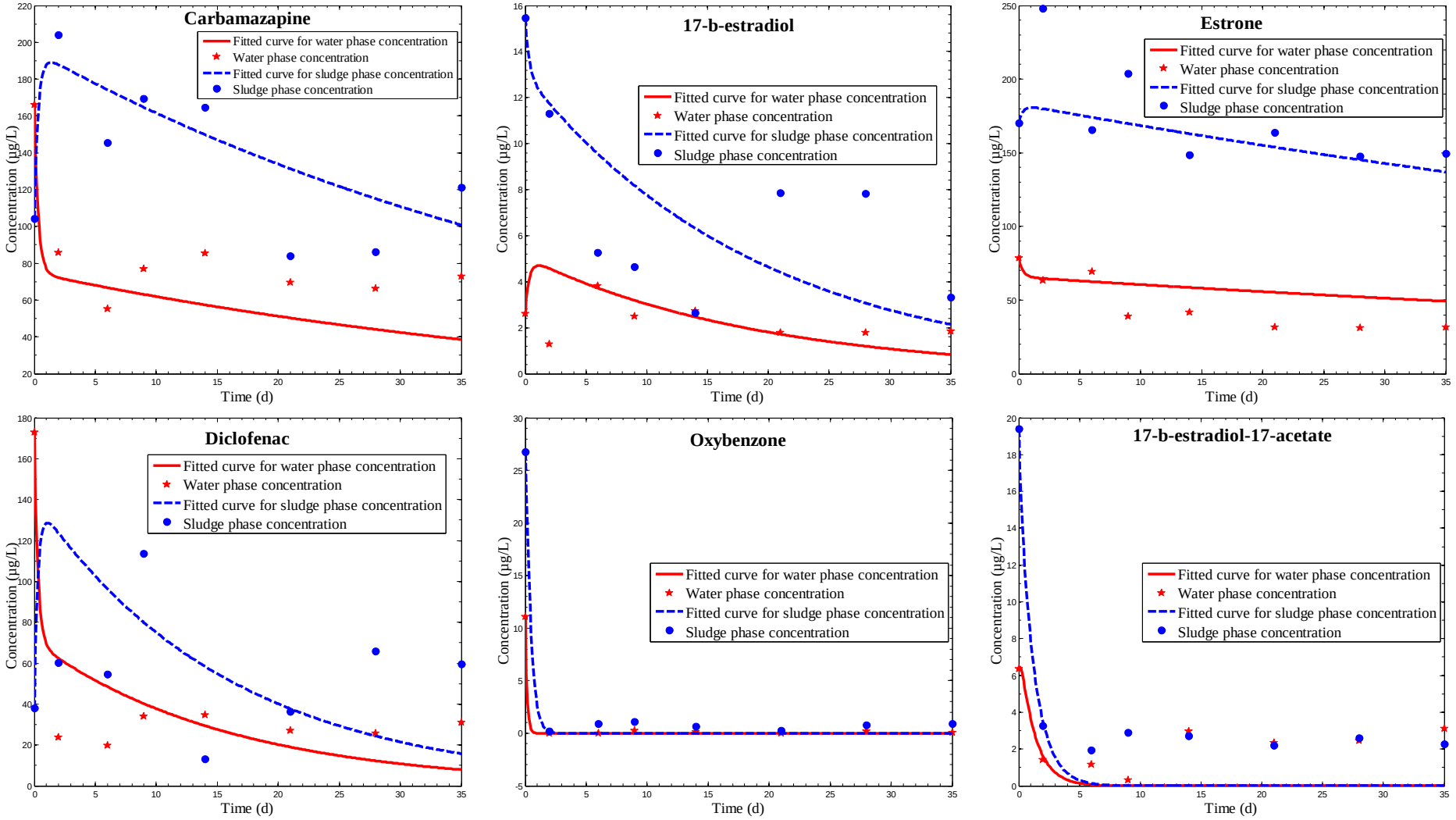

4-tert-butylphenol
Fitted curve for water phase concentration
Water phase concentration
Fitted curve for sludge phase concentration
Sludge phase concentration
Concentration (μg/L)
Time (d)

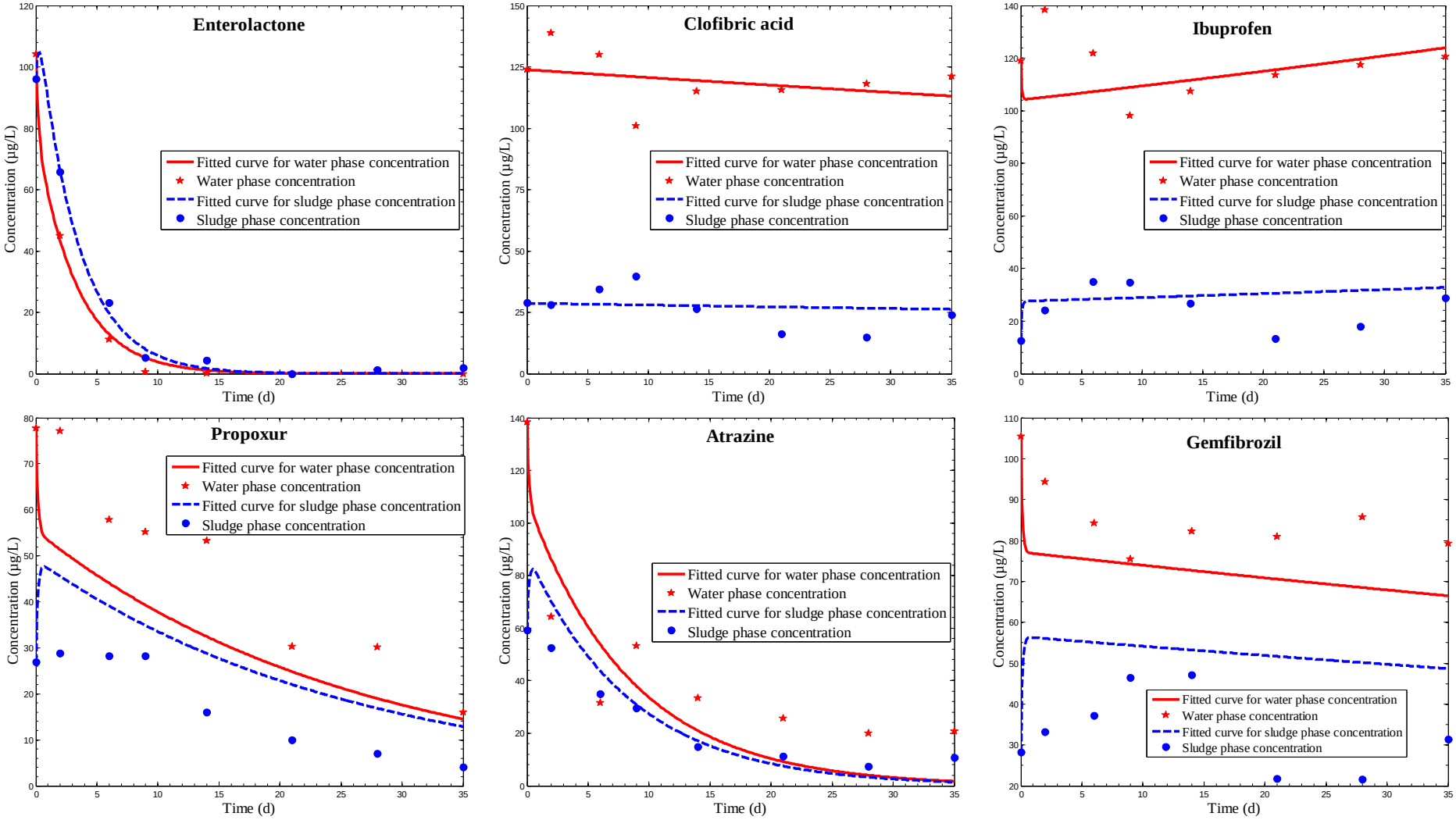

17-a-ethinylestradiol
Fitted curve for water phase concentration
Water phase concentration
Fitted curve for sludge phase concentration
Sludge phase concentration
Concentration (μg/L)
Time (d)


Bisphenol A
Fitted curve for water phase concentration
Water phase concentration
Fitted curve for sludge phase concentration
Sludge phase concentration
Concentration (μg/L)
Time (d)


Benzophenone
Fitted curve for water phase concentration
Water phase concentration
Fitted curve for sludge phase concentration
Sludge phase concentration
Concentration (μg/L)
Time (d)


Estriol
Fitted curve for water phase concentration
Water phase concentration
Fitted curve for sludge phase concentration
Sludge phase concentration
Concentration (μg/L)
Time (d)


Carbamazapine
Fitted curve for water phase concentration
Water phase concentration
Fitted curve for sludge phase concentration
Sludge phase concentration
Concentration (μg/L)
Time (d)
17-b-estradiol
Fitted curve for water phase concentration
Water phase concentration
Fitted curve for sludge phase concentration
Sludge phase concentration
Concentration (μg/L)
Time (d)
Estrone
Fitted curve for water phase concentration
Water phase concentration
Fitted curve for sludge phase concentration
Sludge phase concentration
Concentration (μg/L)
Time (d)
Diclofenac
Fitted curve for water phase concentration
Water phase concentration
Fitted curve for sludge phase concentration
Sludge phase concentration
Concentration (μg/L)
Time (d)
Oxybenzone
Fitted curve for water phase concentration
Water phase concentration
Fitted curve for sludge phase concentration
Sludge phase concentration
Concentration (μg/L)
Time (d)
17-b-estradiol-17-acetate
Fitted curve for water phase concentration
Water phase concentration
Fitted curve for sludge phase concentration
Sludge phase concentration
Concentration (μg/L)
Time (d)


Enterolactone
Clofibric acid
Ibuprofen
Propoxur
Atrazine
Gemfibrozil
Concentration (μg/L)
Time (d)
Fitted curve for water phase concentration
Water phase concentration
Fitted curve for sludge phase concentration
Sludge phase concentration

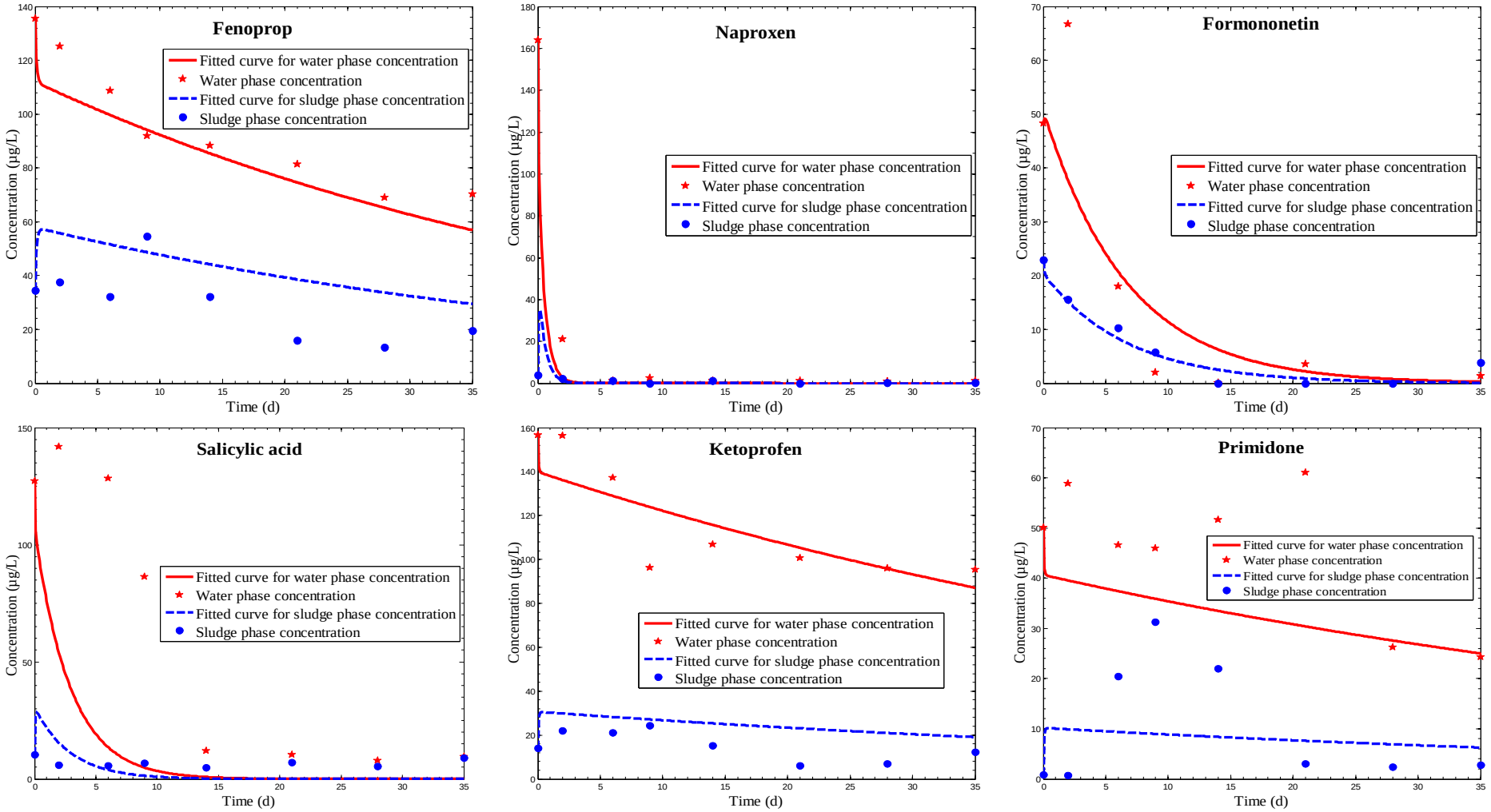


Figure 38. Plots of experimental data and fitting curvesof the two-phase fate model for the selected TrOCs.

6.3 Removal performance of trace organic compounds under anaerobic sludge treatment

6.3.1 Overall removal of trace organic compounds

Biodegradability under anaerobic condition of the TrOCs investigated here represented by k_b varies significantly (Table 24). Some TrOCs (i.e. primidone, amitriptyline, 4-tert-butylphenol, bisphenol A, and 4-tert-octylphenol) are complete recalcitrant to anaerobic digestion ($k_b \approx 0$ L/gVS.d) whilst several others (i.e. formononetin, pentachlorophenol, enterolactone, salicylic acid, 17-β-estradiol-17-acetate, naproxen, and oxybenzone) show significant biodegradability ($k_b > 0.01$ L/gVS.d). The other TrOCs exhibit moderate removal capacity.

TrOC removal was determined after 35 days of anaerobic digestion and compared to literature data obtained from both anaerobic and aerobic conditions (Table 24). Like observations of biodegradation rates, above99% removal was achieved with all phytoestrogens (i.e. formononetin, enterolactone), pentachlorophenol, salycilic acid, 17-β-estradiol-17-acetate, naproxen, and oxybenzone while industrial chemicals showed no observable removal efficiency. For the other classes of TrOCs, the removal efficiency varied over a wide range. Results reported in this study were mostly comparable with data recorded in previous studies under anaerobic condition. On the other hand, the anaerobic digestion is less effective for removing TrOCs when compared to data from aerobic treatment processes reported in the literature (Table 24).

Similar removal efficiency from this investigation and previous studies could be observed with some pharmaceuticals and steroids. Of these TrOCs, however, carbamazepine and diclofenac are exceptions. They have been reported either to be resistant to biological removal under aerobic or anoxic conditions [25, 141, 168, 169] or to be moderately removed only when the seed sludge was long acclimatised [25]. This study, by contrast, shows 48.3% and 88.8% removal of carbamazepine and diclofenac, respectively, after 35 days of anaerobic digestion. The results here suggest that carbamazepine and diclofenac are persistent to aerobic treatment but are amendable to anaerobic biodegradation.

Table 24. Overall removal efficiencies of the selected TrOCs in comparison with reported data under anaerobic and aerobic conditions.

Category	Compound	This study			Literature			
					Anaerobic		Aerobic	
		k_b (L/gVS.d)	$t_{1/2}$ (d)	R (%) after 35 d	R (%)	References	R (%)	References
Pharmaceuticals	Salicylic acid	0.0229	2.0	> 99.0	>99.0	[170]	89.0	[141]
	Ketoprofen	0.0009	51.2	37.7	15.0	[168]	70.5 – 89.0	[141, 169]
	Naproxen	0.1243	0.4	> 99.0	70.0 – 99.0	[25, 168, 171]	40.1 – 78.0	[141, 169]
	Metronidazole	0.0003	137.4	16.2	not available		68.5	[141]
	Primidone	0.0009	50.0	38.5	< 0.1 – 2.0	[168, 172]	12.4 – 95.0	[141, 169]
	Ibuprofen	0.0000	-	< 0.1	<0.1	[168]	96.7 – 99.0	[141, 169]
	Diclofenac	0.0042	11.1	88.8	<0.1 – 60.0	[24, 25, 168, 171]	17.3 – 27.0	[141, 169]
	Gemfibrozil	0.0003	162.8	13.8	not available		91.0 – 99.0	[141, 169]
	Carbamazepine	0.0013	36.7	48.3	<0.1 – 5.0	[24, 25, 168, 172, 173]	< 0.1 – 58.0	[141, 169]
	Amitriptyline	0.0000	-	<0.1	47.0	[168]	78.0 – 97.8	[141, 169]
	Triclosan	0.0027	16.9	76.2	90.0	[168]	44.0 – 91.8	[141, 169]
Steroid hormones	Estriol	0.0005	84.0	25.1	<0.1	[168]	84.0 – 98.2	[141, 169]
	Estrone	0.0018	25.1	62.0	<0.1	[168]	97.0 – 98.0	[141, 169]
	17-α-ethinylestradiol	0.0005	92.4	23.1	15.0	[168]	84.0 – 93.5	[141, 169]
	17-β-estradiol	0.0034	13.4	83.5	60.0	[168]	99.0 – 99.4	[141, 169]
	17-β-estradiol-17-acetate	0.0539	0.9	> 99.0	not available		98.0	[141]

Category	Compound	This study			Literature			
					Anaerobic		Aerobic	
		k_b (L/gVS.d)	$t_{1/2}$ (d)	R (%) after 35 d	R (%)	References	R (%)	References
Pesticides	Clofibric acid	0.0003	140.8	15.8	not available		81.0	[141]
	Fenoprop	0.0013	35.8	49.2	not available		83.0	[141]
	Propoxur	0.0026	18.1	73.8	not available		58.0	[141]
	Pentachlorophenol	0.0171	2.7	> 99.0	>99.0	[174]	83.0	[141, 175]
	Atrazine	0.0078	5.9	98.4	7.0	[168]	4.4 – 36.0	[141, 169]
	Ametryn	0.0036	12.9	84.7	not available		92.0	[141]
Industrial chemicals	4-tert-butylphenol	0.0000	-	< 0.1	not available		91.0	[141]
	Bisphenol A	0.0000	-	< 0.1	0 – 32.0	[168, 176]	90.4	[141]
	4-tert-octylphenol	0.0000	-	< 0.1	not available		96.0	[141]
Phytoestrogen	Formononetin	0.0100	4.6	> 99.0	>99.0	[177]	93.0	[141]
	Enterolactone	0.0202	2.3	> 99.0	not available		92.0	[141]
UV filters	Benzophenone	0.0000	-	< 0.1	not available		99.0	[141]
	Oxybenzone	0.1797	0.3	> 99.0	>99.0	[178]	98.0	[141, 178, 179]
	Octocrylene	0.0000	-	< 0.1	not available		88.0	[141]

Removal efficiency (R), biodegradation rate constant (k_b), and half-life time ($t_{1/2}$).

In this investigation, negligible removal was observed for compounds, including metronidazole, ibuprofen, benzophenone, 4-tert-butylpheol, octocrylene, and bisphenol A. Previous studies examining aerobic conditions, nonetheless, reported moderate to high removal of these TrOCs [141, 169, 176, 179-181]. The difference between anaerobic and aerobic microbes can be a possible explanation for this observation, suggesting higher capacity of degrading TrOCs of aerobic populations. This could be clarified by identifying microbes involved in anaerobic digestion, which was however beyond the scope of this study.

6.3.2 Role of chemical structures

The bioavailability of trace organics during anaerobic conversion depends on not only distinct bacterial community and their synergic effects but also their physicochemical features. Therefore, an assessment of the relative effects of TrOC properties on their removal efficiencies was more focused in order to establish certain applicable generalisations.

Results in this batch test exhibited the varied removal extent by anaerobic biodegradation in both hydrophilic and hydrophobic compounds. Under aerobic conditions, it was reported that the excellent removal were observed in hydrophilic compounds (log $D_{pH=8}$< 3.2) [141, 169, 180]. This is because most of those aerobic studies examined the decrease of TrOC concentrations only in the water phase. As such, two main mechanisms, namely biodegradation in the water phase and sorption from the water phase into solid phase were responsible for this change. On the other hand, the removal of TrOCs in the current investigation was determined from their concentrations in both the water and solid phases. Thus, biodegradation was the only removal mechanism. Since these TrOCs were selected based on their diversity with respect to origins, intended usages, and physicochemical features, it is essential to examine the role of chemical structures in determining their biodegradability under anaerobic sludge treatment.

A detailed examination of the chemical structures and properties of the selected TrOCs was conducted to elucidate their biodegradability under anaerobic condition. When chemical features in terms of the complexity of aromatic rings and properties of functional groups were compared, it is noted that there was no clear association between the nucleus complexity and the elimination capacity of selected TrOCs. This

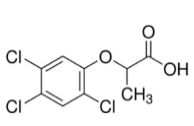
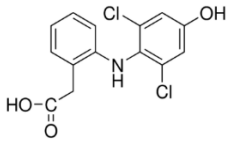
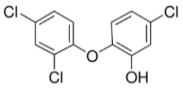
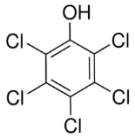
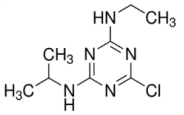
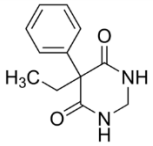
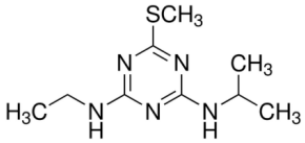
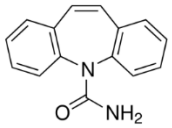
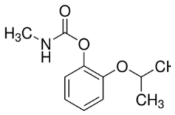
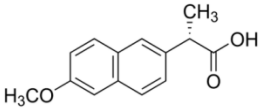
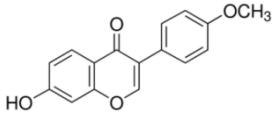
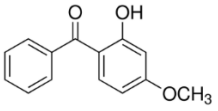
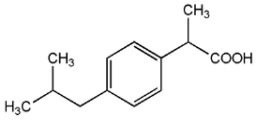
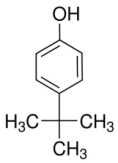
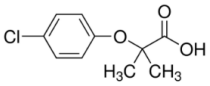
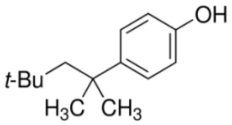
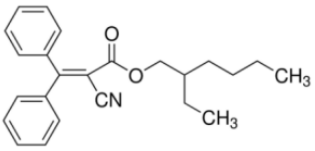
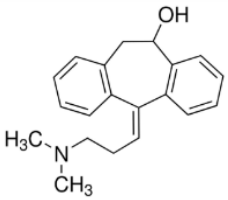
is because the main tendency of bacterial activity is mostly to breakdown any compound from exterior structure, followed by a further nucleus attack.

Considering electron donating (EDG) and electron withdrawing (EWG) groups play a discernible role in electrophilic nucleus orientation, a great deal of research has indicated greater effect of EDG than EWG on making organic compounds more susceptible to biodegradation. The underlying rationale was that EDG is inclined to drive molecules more susceptible to electrophilic attack by oxygenases of aerobic microbes. This apparent correlation was successfully utilised to create feasible frameworks for predicting the removal extent of given sets of TrOCs during the aerobic treatment processes [141, 169, 182]. Nonetheless, this framework is not suitable for assessing the biodegradability of TrOCs under an anaerobic condition.

Table 25 summarises key functional moieties that can influence the removal efficiency of TrOCs in an anaerobic condition.

Anaerobic digestion showed excellent removal of formononetin, oxybenzone, and naproxen (>99%). The inclusion of methoxy group in their structures could be responsible for complete bioconversion of these three compounds. As previously discussed, the readily biodegradability of methanol solvent was demonstrated to enhance methanogenesis in TrOC-spiked bottles. The growth of specific bacterial species selectively working on organic compounds analogous to methanol was consequently stimulated. Therefore, these compounds were simultaneously biotransformed with methanol from the start-up of digestion (i.e. $t_{1/2}$ values of oxybenzone, naproxen, and formononetin are 0.3, 0.4, and 4.6 days, respectively). In a good agreement with the literature, Liu et al. [178] suggested that oxybenzone was more favourably degraded by anaerobic incubation than other redox conditions, achieving its complete removal after 42 days. In a more recent study of UV filters in aquifers, the well degradation under aerobic and anaerobic conditions of this compound were also observed by Liu et al. [179]. In order to enhance the biodegradation of certain organic compounds in the absence of oxygen, the usage of methanol as an electron donor in anaerobic metabolic pathways has been widely investigated in the literature. By means of dehalogenation, a nearly complete elimination of halogenated compounds in methanol-fed anaerobic digesters has been recorded (i.e. more than 99% for hexachlorocyclohexane [183] and 2,4,6-trichlorophenol [184]) compared to that of digesters fed with other electron donors.

Table 25. Relation of functional moieties and the removal efficiency of the selected TrOCs.

Compounds containing chloride groups and/or amine/amide with the effective removal
 Fenoprop  Diclofenac  Triclosan  Pentachlorophenol  Atrazine  Primidone  Ametryn  Carbamazepine  Propoxur
Compounds containing methoxy groups with the high effective removal
 Naproxen  Formononetin  Oxybenzone
Compounds containing long alkyl chain with low removal efficiency
 Ibuprofen  4-tert-butylphenol  Clofibric acid  4-tert-octylphenol  Octocrylene  Amitriptyline

In this investigation, a varied range of removal efficiencies was observed with chlorinated TrOCs in the range of 15.8% to more than 99% (Table 25). The increasing order of removal was observed with the greater extent of chlorination, with the excellent removal of pentachlorophenol (more than 99%). This observation is in a good agreement with the literature [175]. The high biodegradability this class of compounds under anaerobic condition can be attributed to anaerobic metabolism through the dehalogenation pathway. By contrast, aerobic microorganisms initialize the degradation pathway of halogenated compounds via oxidation from other co-

existing functional groups [169]. This observation suggests that less halogenated compounds are more readily biotransformed with oxygen exposure. On the other hand, the inclusion of alkyl chain in chloride-containing compounds could affect their conversion pathway, resulting lower removal efficiencies, for which clofibric acid was a particular example (15.8% elimination).

The presence of amide/amine groups in TrOC chemical structures can affect their removal under anaerobic condition. Some of TrOCs bearing amine/amide have been shown to be poorly removed in previous studies. Carbamazepine was, partly removed with efficiency of 48.3% in this investigation, considered as a typical example of very persistent xenobiotic compounds regardless of redox conditions [25, 168, 172, 173]. The moderate removal (38.5%) was here recorded for primidone, which was found to pass through soil to groundwater under anaerobic condition by Ternes et al. [172]. Higher removal of such compounds as diclofenac and atrazine (60% and 85%, respectively) may be also due to the co-existence of the chloride group in their chemical structures, which was poorly removed by aerobic MBR treatment [141, 169]. Propoxur and ametryn also presented high removal efficiencies (i.e. 73.8% and 84.7%, respectively) as reported [141, 169], showing their highly bioavailability to any redox conditions. Interestingly, amitriptyline bearing both chloride and amine groups showed highly persistent to anaerobic degradation. This behaviour could be ascribed to the existence of long alkyl group.

Negligible removal efficiency ($k_b \approx 0$ L/gVS.d) was observed with some compounds, including ibuprofen, 4-tert butylphenol, 4-tert-octylphenol, octocrylene, bisphenol A, amitriptyline, and benzophenone. Except for benzophenone, which has no functional group, the inclusion of alkyl group (Table 25) in chemical structure of other compounds was responsible for their poor biodegradation. It should be noted that more than 70% dissipation of octocrylene was obtained after 77 incubation days with anaerobic consortium in a native aquifer [179]. These contrasting observations here may be associated to the distinct indigenous bacteria populations available in aquifers and anaerobic sludge. To our best understanding, while investigations regarding the biodegradation of 4-alkylphenols under anaerobic condition have been still limited. Their great attenuation [180] and involved biodegradation pathways [181] have been only studied in the context of aerobic processes. The inclusion of the alkyl side chain was probably responsible for their poor removal. In the literature, a

negative effect of long alkyl chain on the biodegradation rate was demonstrated in case of phthalate esters [185]. The concentration of bisphenol A seemed to be stable during this anaerobic conversion. Bisphenol A was previously reported to be insignificantly degraded by anaerobic consortium at efficiency of 37% [168] and even persistent to anaerobic degradation [176]. A significant decrease in concentration of this compound, by contrast, has been found under different aerobic treatment processes [176, 180].

For steroid hormones, it is noted that the high biodegradation (>83.5%) was achieved with 17-β-estradiol, while estrone exhibited the lower removal efficiency (62.0%) at day 35. This observation could be attributed to possible oxidation of 17-β-estradiol to estrone, which was observed in anaerobic sediments [23, 186]. The various extent of biodegradation of members in this group has been widely but contrastingly reported. A substantial level of estrone, and 17-β-estradiol was still detected in UASB effluent whilst any type of sludges did not show the removal capacity of 7-α-ethinylestradiol [126, 186]. By contrast, Carballa et al. [25] reported significant decreases in sum concentrations of estrone and 17-β-estradiol (85%), and 17-α-ethinylestradiol (60%), whose removal in this batch test was reported at 45%. Little was known about the anaerobic biodegradation of 17-β-estradiol-17-acetate, which was here observed to be effectively degraded (> 99%).

The existence of functional groups in chemical structures of TrOCs determined their various biodegradation rates throughout the anaerobic conversion. In particular, while the inclusion the halogen, methoxy and amine/amide groups rapidly simulated the anaerobic degradation/transformation of TrOCs; the alkyl group was responsible for their biological recalcitrance to anaerobic treatment.

6.4 Fate of trace organic compounds in sludge matrix under anaerobic condition

The distribution of TrOCs in the water and solid phases during anaerobic treatment can be presented by k_p obtained from the two-phase fate model. Within anaerobic treatment of sewage sludge, both k_p values reported here and those reported in the literature showed comparable sorption properties of respective TrOC groups. Negligible adsorption (k_p< 0.1 L/g) of pharmaceuticals such as naproxen, ibuprofen, and diclofenac to the solid phase reported in the current investigation is consistent with previous studies in case of primary and secondary sludge [187] and of digested

sludge [188]. A correspondence between high k_p values and their high hydrophobicity nature was only found in steroid hormones, of which 17-β-estradiol-17-acetate was an exception. For the other compounds in this group, there is an agreement of their high adsorption potential between this study and previous studies, estimated k_p values here were lower than values reported from the literature [189].

In the initial period of spiking, a fraction of investigated compounds was adsorbed onto solid particles. Different sorption properties onto sludge particles were found for these trace organics over the experimental time.

It was expected that the distribution of TrOCs was in a relation with their physicochemical properties, particularly their hydrophobicity (log *D*). Figure 39, however, shows a weak correlation between log *D* (pH = 8) and log k_p of the TrOCs investigated here (R^2 = 0.427). The same observation was stated in the literature although several previous attempts have been made to analyse the relationship between log *D* and adsorption of substances into different environmental samples, including activated sludge [190], primary and secondary sludge [187], and digested sludge [188]. Even though pK_a at the ambient pH was considered in modelling, Carballa et al. [188] found a significant high deviation modelled values in case of iopromide, sulfamethoxazole and roxithromycin. One possible explanation is that sorption behaviour while depends on physicochemical properties of involved chemicals and solid compositions, is still affected by other conditions, including pH, temperature, and ion strength [191]. Sorption behaviour was also found to be dependent on initial concentrations applied as reported in case of bisphenol A [189]. Another reason is that biodegradation factor was taken into account. As such, in view of diverse TrOC groups selected in this anaerobic treatment study, their hydrophobicity and distribution in two phases of sludge was then examined along with different biodegradation rate.

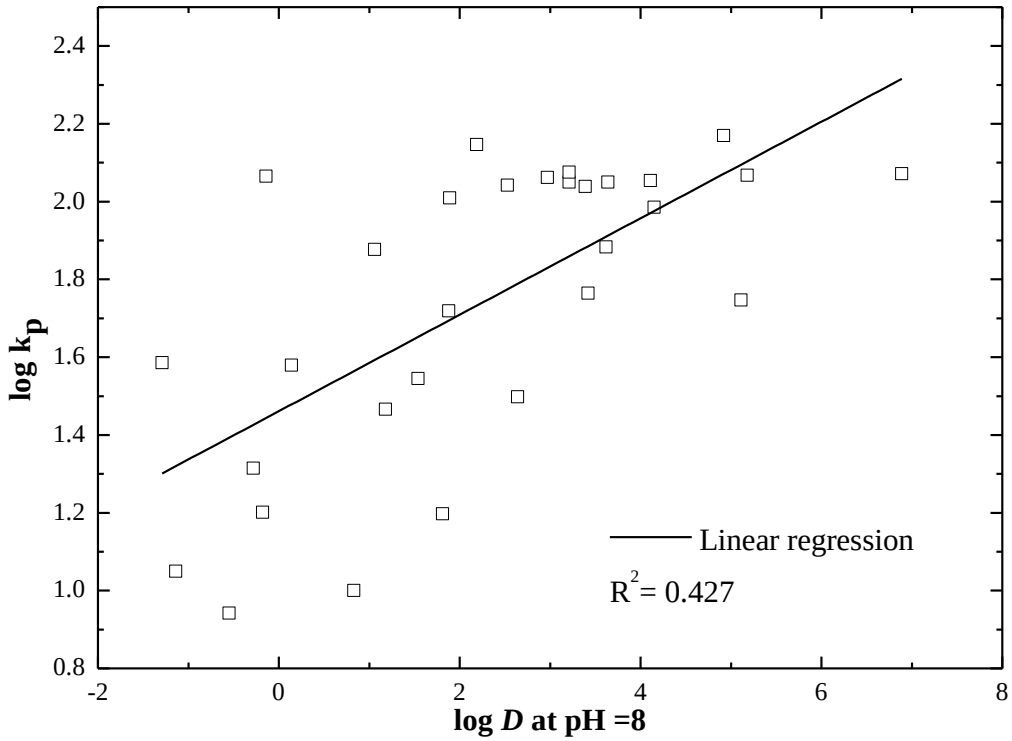


Figure 39. Correlation between log *D* (pH = 8) and log k_p of the selected TrOCs.

The biodegradability of TrOCs is classified based on biodegradability rate (k_b) as illustrated in Figure 40. For compounds with negligible to low removal rates ($k_b \leq$ 0.001 L/gVS.d), their hydrophobicity (log *D* at pH = 8) and their partition (k_p) in sludge were comparable. There was a moderate relationship (R^2 = 0.715) between them as illustrated in Figure 41. In this occasion, the biodegradation rate had no effect on their distribution in sludge system. Instead, the adsorption tendency of these TrOCs was dependent on their hydrophobicity (known as log *D*). High concentrations in the solid phase (k_p> 0.1 L/g) of some compounds such as octocrylene and 4-tert-butylphenol could be attributed to the inclusion of the alkyl group, which referred to biological persistent TrOCs (Section 6.3.2). Similarly, Ismail et al. [192] observed an increase of sorption capacity according to increasing alkyl chain length in case of quaternary ammonium compounds. Therefore, the fate in two-compartment sludge of biological persistent compounds could be predicted by means of their hydrophobicity without any effect of biodegradability.

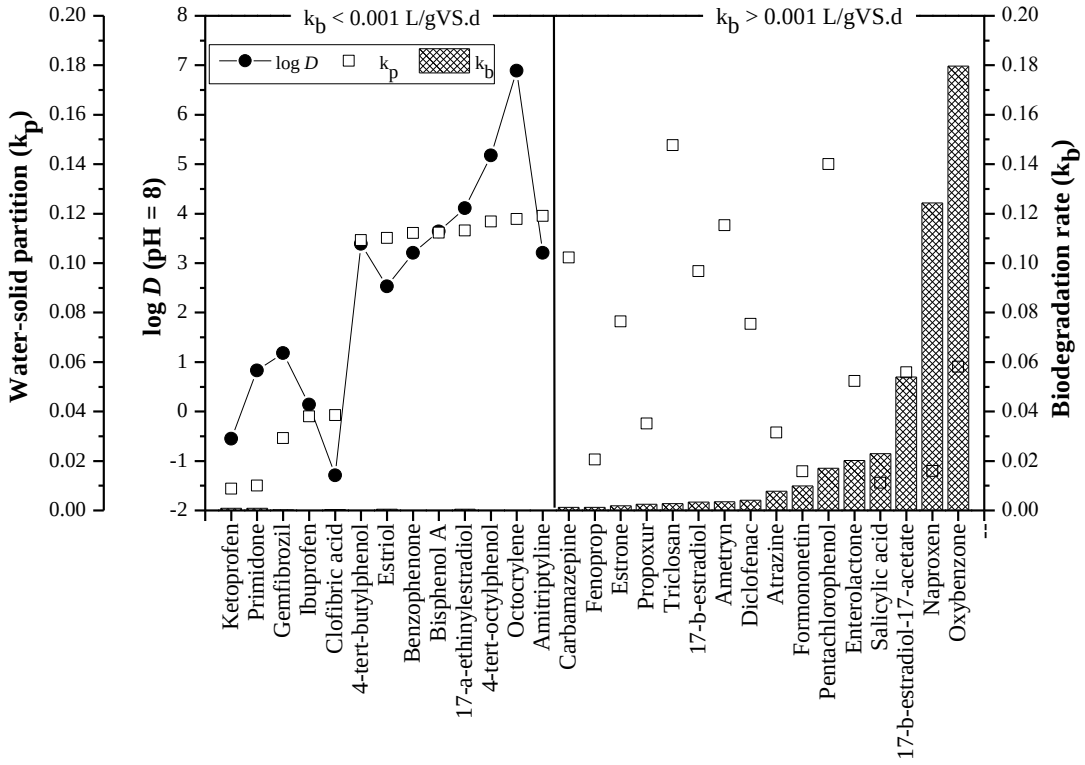
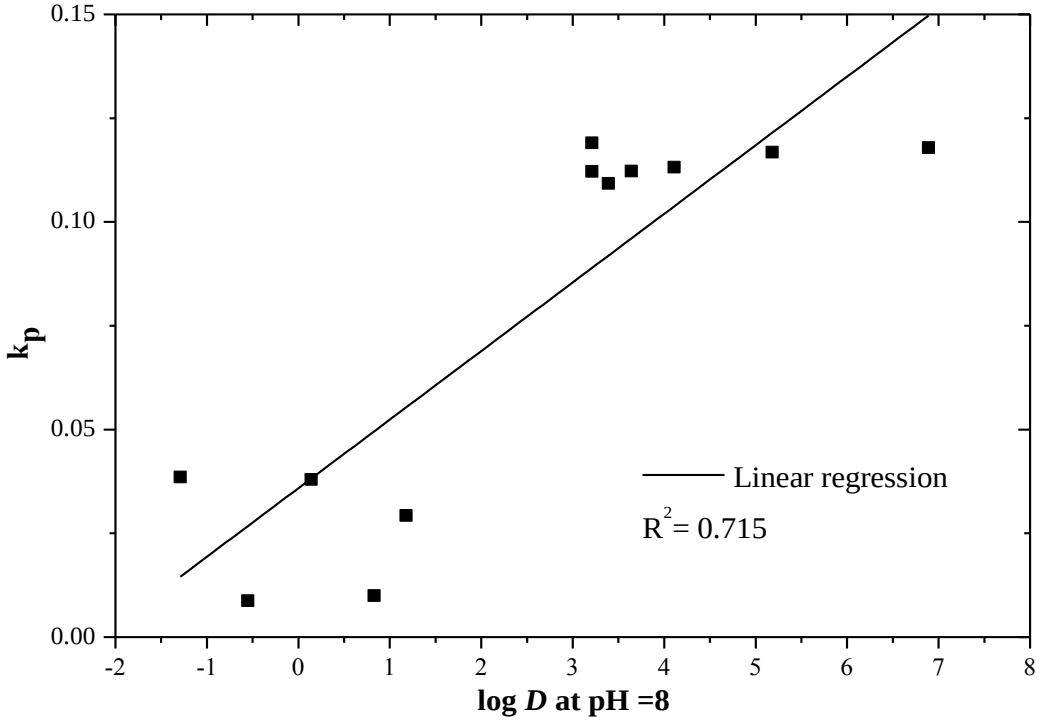
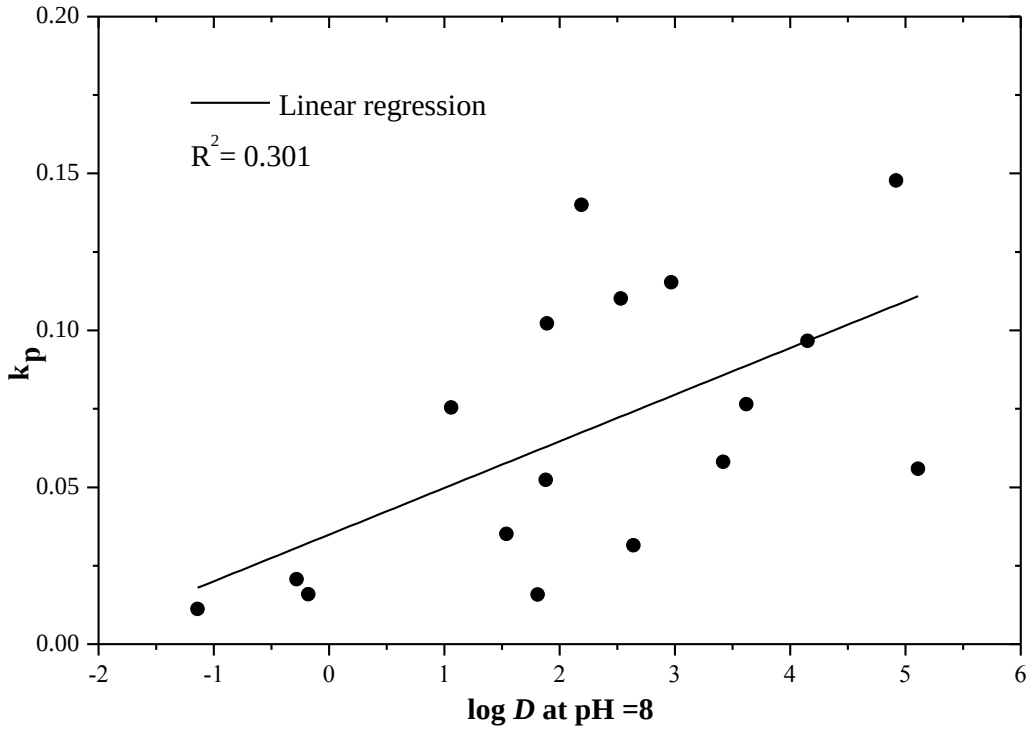


Figure 40. Relation among biodegradation rate constant (k_b, L/gVS.d) water-solid partition (k_p, L/g) and log *D* (pH =8) of the selected TrOCs.

For the other compounds, which are amenable to anaerobic digestion (k_b> 0.001 L/gVS.d), their biodegradability rate and sorption properties played an important role in governing their elimination in anaerobic sludge treatment. There was a weak correlation between log *D* (pH = 8) and k_p of these TrOCs (Figure 41) due to low correlation coefficient (R^2 = 0.301). Log *D* values should be ruled out from explaining their fate in two-compartment sludge under anaerobic condition in this case.



a. k_b< 0.001 L/gVS.d



b. k_b> 0.001 L/gVS.d

Figure 41. Correlation of log *D* (pH = 8) and k_p of compounds showing low (a) and high (b) biodegradability rates (k_b).

6.5 Summary

System stability of this anaerobic sludge digestion was confirmed by evaluating temporal profile of control parameters and the CH_4 production. Firstly, favourable operating conditions in case of pH and buffer capacity (i.e. close to neutral pH and high alkalinity of more than 4000 $mgCaCO_3/L$, respectively) were observed in TrOC-spiked bottles throughout the digestion time. Secondly, results of CH_4 production obtained from experimental data and from the first order and modified Gompertz kinetic models showed mostly similar CH_4 generation between bottles with and without TrOCs. Their differences, including higher CH_4 production from day4 to 9 and longer lag time of around 1.6 days in spiked bottles, resulted from the usage of methanol as the solvent in TrOC preparation. A steady state of methanogenesis was then created in TrOC-spiked bottles after 1.6 days.

Their various removal efficiencies were consistently related to their molecular properties in terms of functional groups rather than other physicochemical properties. Compounds with the inclusion of halogen, methoxy and amine/amide groups exhibited an effective removal while lower to negligible elimination efficiencies were observed in compounds possessing long alkyl group under same conditions. In this study, the main removal mechanism of investigated TrOCs from sludge matrix was anaerobic biodegradation. In terms of TrOC removal, the distribution of selected TrOCs in water and solid phase was examined using the two-phase fate model. Regarding compounds possessing high biodegradability ($k_b > 0.001 L/gVS.d$), k_p values instead of their hydrophobicity (namely log *D*) calculated from the proposed two-phase fate model demonstrated a relation to their partition in the sludge phase. For biologically persistent compounds to anaerobic conversion ($k_b < 0.001 L/gVS.d$), sorption properties, which can be predicted by log *D* values, were responsible for their fate of in sludge matrix.

7 CONCLUSIONS AND RECOMMENDATIONS

7.1 Conclusions

The treatment performance of sewage sludge under anaerobic condition was investigated in this study. Firstly, a critical review on the up-to-date literature of the AD process was concentrated on two key aspects, namely CH_4 production enhancement and TrOC removal efficiencies. Secondly, a series of batch tests with respect to both aspects were conducted to envision the potential of glycerol as a co-substrate in enhancing CH_4 yields, and to widen the understanding of the capacity in eliminating diverse TrOCs in sewage sludge under anaerobic condition. The main conclusions were summarised as following.

In the first series of batch-mode biomethane potential (BMP) tests, the potential of CH_4 yields and process stability of each sewage sludge and glycerol was tested based on their characteristics. Data from experimental observations indicated a significant improvement in terms of yielding $CH_4$and system stability by the buffer supplement ($NaHCO_3$). Compared to the non-buffed bottles, higher performance in producing CH_4 was recorded in bottles with addition of 15 and 30 mM $NaHCO_3$. Estimated lag times from kinetic analyses of the first-order and modified Gompertz models also gave an evidence of such stimulation of CH_4 generation by buffer addition. It is noteworthy that buffer concentrations applied in this study were still not adequate to initialise CH_4 production. This instability could be attributed to insufficient methanogenic activity.

BMP of raw primary sludge was alternatively tested by using 1/1 ratio rather than 1/9 ratio of I/S by volume. Stability in methanogenesis of raw primary sludge was achieved during this batch test due to favourable anaerobic conditions (i.e. neutral pH and high buffer capacity), revealing an important role of I/S ratio. Both experimental results and kinetic analysis data demonstrated a great potential of raw primary sludge in CH_4 yield, low CH_4 production rate and long lag phase made it unfavourable for a well-established anaerobic digester though. The supplement of substrates rich in readily biodegradable part was prospectively considered to accelerate the methanogenesis of raw primary sludge in particular and sewage sludge in general.

Another series of BMP tests of glycerol were carried out with the adequate inoculum supplementation. Evaluation of control parameters among three types of glycerol at different concentrations showed a stable function of anaerobic conversion. It could be expected from the high experimental and predicted CH_4 potential yields that any glycerol should be considered a feasible co-substrate for sewage sludge. Their high soluble organic fraction and solubility also supported this expectation.

Data from batch tests of anaerobic co-digestion of raw primary sludge and glycerol pointed out that the addition of 0.5% and 1.0% of glycerol into raw primary sludge was satisfactory in terms of daily and cumulative CH_4 production within the start-up stage. This improvement resulted from the enhancement of organic source with regard to COD_s, the highly biodegradability and solubility of glycerol. On the other hand, certain characteristics of digestates, typical of higher COD_s, acidic pH and inadequate alkalinity as well as short methanogenic duration, indicated the incomplete anaerobic biodegradation for the long-term operation. These findings indicated the great effects of I/S ratio and buffer supplementation on system stability and then the ultimate CH_4 potential.

The last batch-mode BMP tests were performed to evaluate the removal efficiency of selected TrOCs by anaerobic treatment. In terms of system stability, experimental results and kinetic data of parameter variations and CH_4 yields indicated a steady state of methanogenesis, which was created after 1.6 days in TrOC-spiked bottles. Biodegradation was the main mechanism for the removal of TrOCs during this AD of sludge. The extent of biodegradability of TrOCs was related to their molecular properties in terms of functional groups rather than other physicochemical properties. Compounds with the inclusion of halogen, methoxy and amine/amide groups exhibited an effective removal while low to no removal efficiencies were observed in compounds possessing long alkyl group under same conditions. In general, the distribution of selected TrOCs in sludge system under anaerobic conditions exhibited a weak correlation to their hydrophobicity (log D). Particularly considering biologically compounds persistent to anaerobic conversion, their water-solid partition (k_p) can be predicted by their log D. In case of TrOCs amenable to anaerobic conversion, k_p calculated from the proposed two-phase fate model are more appropriate to describe their fate in sludge matrix than log D values.

7.2 Recommendations for future studies

Some suggestions for future studies were made over this research course.

Given the great effects of buffer supplement and I/S ratio on the system stability, it is highly recommended to investigate appropriate I/S ratios and buffer concentrations for a stable performance of methanogenic conversion, after which the ultimate CH_4 yields of co-substrate mixtures will be attained.

It would be also necessary to evaluate the limit glycerol concentration beyond which a shock of organic loading will happen under steady state of anaerobic processes. Further, BMP of anaerobically co-digesting sewage sludge with other potential organic materials, beverage rejects and food waste, as examples should be extensively investigated. These findings at laboratory-scale batch mode are expected to be valuable for larger scale operations in WWTPs in terms of economic benefits and time saving.

Compounds possessing the methoxy group in their chemical structures effectively eliminated with the presence of methanol under anaerobic condition, suggesting in some extent a positive role of methanol in anaerobic biodegradation of TrOCs. This suggestion deserves more studies to comprehensively examine a real effect of co-digestion with readily biodegradable substrate on anaerobic metabolism of a particular compound.

The observed significant variation from negligible to excellent removal capacity of the anaerobic sludge treatment requires more understanding of main factors, which govern the elimination of specific chemicals. The long-term exposure of inoculum to TrOCs has been considered as an example. This aspect, however, has not been included in this study. Such acclimatisation could be possibly envisioned through more batch experiments using TrOC-acclimatised anaerobic sludge. Quantification and identification of associated anaerobic populations responsible for degrading TrOCs could be also endorsed in a broader context.

Modifications of the applied two-phase fate kinetic model should be made to develop a novel mathematic model. Considering all possible factors associated to the dynamics of TrOC concentrations over the digestion time, this model is expected to more precisely predict their fate in sludge matrix under anaerobic condition, and their attenuation subsequently.

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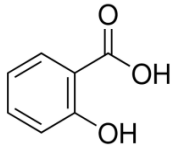
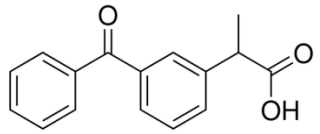
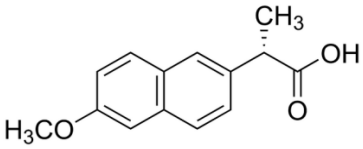
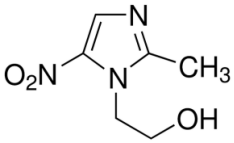
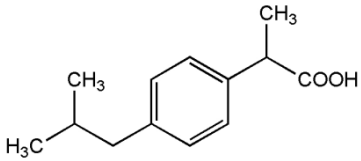
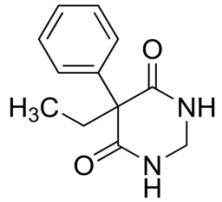
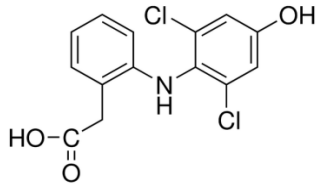
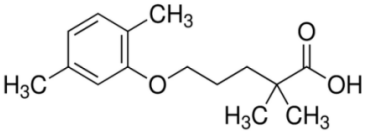
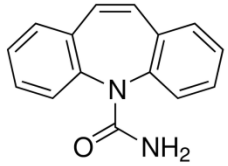
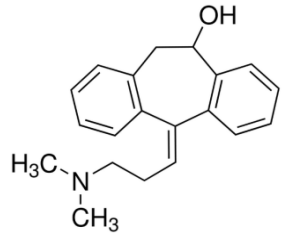
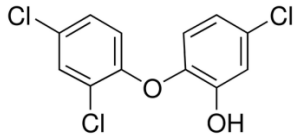
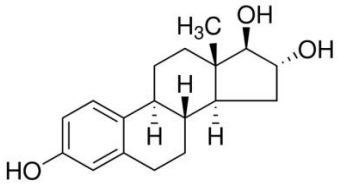
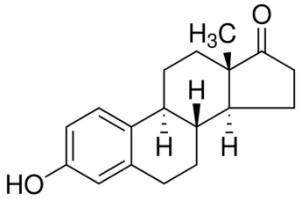
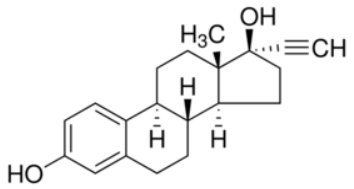
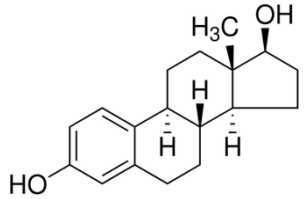
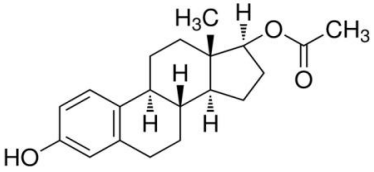
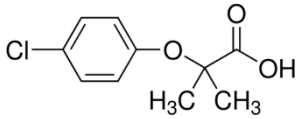
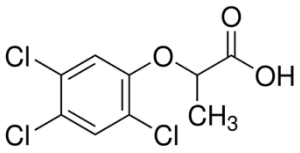
APPENDIX

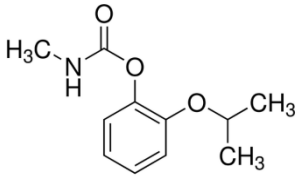
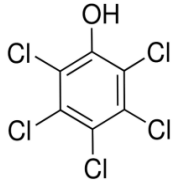
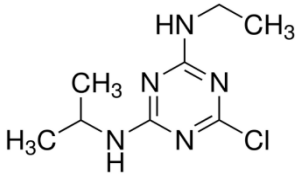
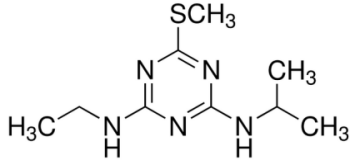
Table 26. Selected TrOCs and their relevant physicochemical properties.

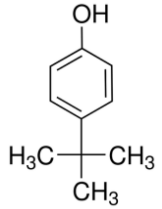
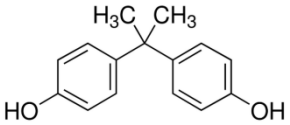
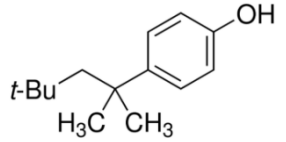
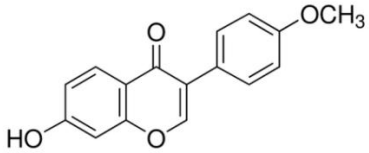
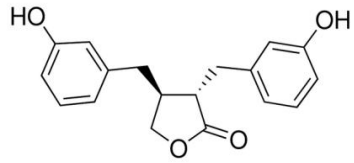
Group	Compound	Molecular formula	Molecular weight (g/mol)	Log *D* at pH = 8	Henry's Law constant at 25 °C ($atm.m^3/mol$)	Chemical structures
Pharmaceuticals	Salicylic acid	$C_7H_6O_3$	138.12	–1.14	1.42×10^{-8}	
	Ketoprofen	$C_{16}H_{14}O_3$	254.30	–0.55	1.92×10^{-13}	
	Naproxen	$C_{14}H_{14}O_3$	230.26	–0.18	2.07×10^{-12}	
	Metronidazole	$C_6H_9N_3O_3$	171.15	–0.14	6.08×10^{-12}	

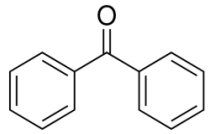
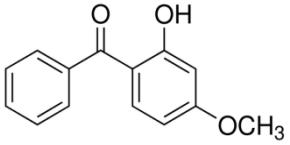
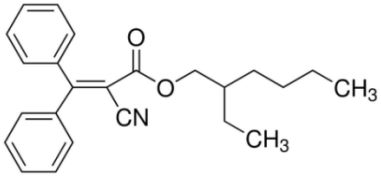
Group	Compound	Molecular formula	Molecular weight (g/mol)	Log *D* at pH = 8	Henry's Law constant at 25 °C ($atm.m^3/mol$)	Chemical structures
	Ibuprofen	$C_{13}H_{18}O_2$	206.28	0.14	5.54×10^{-12}	
	Primidone	$C_{12}H_{14}N_2O_2$	218.25	0.83	1.16×10^{-14}	
	Diclofenac	$C_{14}H_{11}Cl_2NO_2$	296.15	1.06	2.69×10^{-11}	
	Gemfibrozil	$C_{15}H_{22}O_3$	250.33	1.18	1.83×10^{-11}	
	Carbamazepine	$C_{15}H_{12}N_2O$	236.27	1.89	9.41×10^{-12}	

Group	Compound	Molecular formula	Molecular weight (g/mol)	Log *D* at pH = 8	Henry's Law constant at 25 °C ($atm.m^3/mol$)	Chemical structures
	Amitriptyline	$C_{20}H_{23}N$	277.40	3.21	1.24×10^{-10}	
	Triclosan	$C_{12}H_7Cl_3O_2$	289.54	4.92	9.49×10^{-6}	
Steroid hormones	Estriol	$C_{18}H_{24}O_3$	288.38	2.53	1.75×10^{-11}	
	Estrone	$C_{18}H_{22}O_2$	270.37	3.62	9.61×10^{-10}	

Group	Compound	Molecular formula	Molecular weight (g/mol)	Log *D* at pH = 8	Henry's Law constant at 25 °C ($atm.m^3/mol$)	Chemical structures
	17-α -ethinylestradiol	$C_{20}H_{24}O_2$	269.4	4.11	3.74×10^{-10}	
	17-β-estradiol	$C_{18}H_{24}O_2$	272.28	4.15	1.17×10^{-9}	
	17-β-estradiol-17-acetate	$C_{20}H_{26}O_3$	314.42	5.11	2.15×10^{-9}	
Pesticides	Clofibric acid	$C_{10}H_{11}ClO_3$	214.65	–1.29	2.91×10^{-10}	
	Fenoprop	$C_9H_7Cl_3O_3$	269.51	–0.28	4.72×10^{-12}	

Group	Compound	Molecular formula	Molecular weight (g/mol)	Log *D* at pH = 8	Henry's Law constant at 25 °C ($atm.m^3/mol$)	Chemical structures
	Propoxur	$C_{11}H_{15}NO_3$	209.24	1.54	5.26×10^{-7}	
	Pentachlorophenol	C_6HCl_5O	266.34	2.19	1.82×10^{-7}	
	Atrazine	$C_8H_{14}ClN_5$	215.68	2.64	5.22×10^{-8}	
	Ametryn	$C_9H_{17}N_5S$	227.33	2.97	3.67×10^{-9}	

Group	Compound	Molecular formula	Molecular weight (g/mol)	Log *D* at pH = 8	Henry's Law constant at 25 °C ($atm.m^3/mol$)	Chemical structures
Industrial chemicals	4-tert-butylphenol	$C_{10}H_{14}O$	150.22	3.39	7.51×10^{-6}	
	Bisphenol A	$C_{15}H_{16}O_2$	228.29	3.64	1.34×10^{-9}	
	4-tert-octylphenol	$C_{14}H_{22}O$	206.32	5.18	8.67×10^{-6}	
Phytoestrogen	Formononetin	$C_{16}H_{12}O_4$	268.26	1.81	2.91×10^{-10}	
	Enterolactone	$C_{18}H_{18}O_4$	298.33	1.88	8.07×10^{-13}	

Group	Compound	Molecular formula	Molecular weight (g/mol)	Log *D* at pH = 8	Henry's Law constant at 25 °C ($atm.m^3/mol$)	Chemical structures
UV filters	Benzophenone	$C_{13}H_{10}O$	182.22	3.21	1.31×10^{-6}	
	Oxybenzone	$C_{14}H_{12}O_3$	228.24	3.42	1.22×10^{-8}	
	Octocrylene	$C_{24}H_{27}N$	361.48	6.89	3.38×10^{-9}	

All values were sourced from Scifinder Scholar database (ACD/Labs)