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Semi-aerobic fermentation as a novel pre-treatment to obtain VFA and increase methane yield from primary sludge

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Abstract

There is a growing trend to consider organic wastes as potential sources of renewable energy and value-add products. Fermentation products have emerged as attractive value-add option due to relative easy production and broad application range. However, pre-fermentation and extraction of soluble products may impact down-stream treatment processes, particularly energy recovery by anaerobic digestion. This paper investigates primary sludge pre-fermentation at different temperatures (20, 37, 55, 70ºC), treatment times (12, 24, 48, 72h), and oxygen availability (semi-aerobic, anaerobic); and its impact on anaerobic digestion. Pre-fermentation at 20 and 37 ºC succeeded for VFA production with acetate and propionate being major products. Pre-fermentation at 37, 55 and 70 ºC resulted in higher solubilisation yield but it reduced sludge methane potential by 20%. Under semi-aerobic conditions, pre-fermentation allowed both VFA recovery (43 $gCOD_{VFA}\,kg^{-1}VS$) and improved methane potential. The latter phenomenon was linked to fungi that colonised the sludge top layer during pre-fermentation.

Keywords

Fermentation; Anaerobic digestion; Sewage sludge; Temperature; Fungi

1 Introduction

Municipal wastewater treatment is a core requirement of urban populations and results in generation of large amounts of sewage sludge where wastewater pollutants such as organic matter, nutrients, heavy metals, and pathogens are collected and concentrated. Sewage sludge management is a major issue since up to one-half of the costs of operating municipal waste water treatment plants are associated with sludge treatment and disposal (Lens, 2004). Sewage sludge is conventionally anaerobically digested to recover energy, but there is a growing trend to use sludge as a feedstock in other value-add processes (Zacharof & Lovitt, 2013).

Anaerobic digestion (AD) is a series of biochemical processes where organic matter is converted to biogas by a complex microbial community. The methane content of biogas is an important source of renewable energy, and generally AD processes are net-energy producing rather than net-energy consuming processes. AD also provides avenues to produce a variety of value-add products such as volatile fatty acids (VFA) (i.e. acetate, propionate, and butyrate). VFA are intermediate products during the AD process, thus they are being produced and consumed simultaneously. However, different strategies such as shortening digestion times or decoupling the biochemical reactions involved in the fermentation and methanogenic steps can promote the accumulation of VFA, which can be harvested.

VFA have several potential applications within the wastewater treatment plant (WWTP); for instance, VFA can be used to aid biological nutrient removal, replacing expensive carbon sources, such as methanol (Münch et al., 1999). VFA also have stand-alone value as commodity chemicals or pre-cursors used in the production of renewable plastics and biotextiles (Zacharof & Lovitt, 2013). However, the desired VFA profile is highly dependent on its subsequent application, either within the WWTP or the commodity value. Within WWTP applications, the preferred VFA for denitrification is acetate followed by butyrate and

propionate (Elefsiniotis & Wareham, 2007; Gali et al., 2006), whereas biological phosphorus removal processes typically require an acetate to propionate ratio ranging from 0.25 to 0.75 (Broughton et al., 2008; Yuan et al., 2012). Other bioprocesses, such as the production of bioplastics, require different VFA profiles depending on the desired polymers, for example acetate and butyrate are preferred for polyhydroxybutyrate (PHB) production, while propionate is required when producing polyhydroxyvalerate (PHV) (Shen et al., 2014). Pre-fermentation of primary sludge (PS) has been studied previously with yields varying from 0.1 – 0.4 gVFA g^{-1}VS (Ahn & Speece, 2006; Cokgor et al., 2009; Eastman & Ferguson, 1981; Ucisik & Henze, 2008). Nonetheless, little attention has been paid to the impact of a pre-fermentation step and VFA extraction on down-stream processes, particularly the energy production from AD. This is particularly important when considering the WWTP as an integrated process, since extracting VFA will decrease the amount of organic matter fed to AD, potentially decreasing the energy recovered. There is a need for research to determine if the benefits of VFA production and extraction from primary sludge outweigh the potential loss in methane value. Optimal configurations will be influenced by two main factors: (i) the cost (capital investment and operating expenses) of the extraction process and the revenues obtained from VFA use or sale; and (ii) the impact on methane production (Astals et al., 2015).

The aim of the present study is to evaluate the impact of a pre-fermentation step on subsequent methane yield from primary sludge. The pre-fermentation conditions considered different temperatures (20, 37, 55, and 70 ºC), fermentation periods (12, 24, 48, and 72 h), and oxygen availability (semi-aerobic or anaerobic conditions). The anaerobic biodegradability after pre-fermentation was evaluated using biochemical methane potential (BMP) tests and mathematical modelling.

2 Materials and Methods

2.1 Substrate and inoculum

Primary sludge (PS) was obtained from a municipal WWTP in Queensland (Australia). PS was collected after being thickened by centrifugation and before being mixed with waste activated sludge and fed into the mesophilic AD treatment. PS was pre-fermented immediately after collection. The solid fraction of the pre-fermented sludge was stored at 4 ºC prior to BMP testing (max. 4 days). A second batch of PS was collected from the same municipal WWTP to replicate and validate the results obtained from the first pre-fermentation trial (see details below). Table 1 summarises the main characteristics of the two sets of PS used in this study.

Inoculum for the BMP tests was collected from the same WWTP. The inoculum was taken from a 5,500 m^3 digester that treats mixed sewage sludge (50% primary and 50% secondary sludge on VS-basis) at a hydraulic retention time of 23-24 days and a temperature of 35-37°C. The inoculum was degassed at 37 ºC for 4 days prior to utilisation. A second batch of inoculum was collected from the same location and degassed at 37 ºC for 4 days to replicate and validate the results obtained from one of the pre-fermentation conditions (20 ºC semi-aerobic conditions).

2.2 Experimental set-up

2.2.1 Pre-fermentation experiments

The experimental set-up consisted of two separate steps. First step was pre-fermentation, where 100 g of fresh PS were added to 300 mL glass bottles under semi-aerobic or anaerobic conditions. Semi-aerobic conditions were carried out by leaving the bottles open to the environment, while anaerobic conditions were ensured by flushing the headspace of bottles with N_2 (99.9%), and sealing each bottle with a rubber septum and a screw cap. The first set

of experiments was performed anaerobically at 20, 37, 55, and 70 ºC, and semi-aerobically at 20 ºC with four treatment times at each temperature: 12, 24, 48 and 72 h. All experiments were performed without agitation. All tests were conducted in duplicate. A second set of experiments were performed under semi-aerobic conditions at 20 °C only with treatment times of 24, 48, 72 and 96 h. The second set of semi-aerobic experiments was also performed in duplicate.

Destructive sampling was used, where serum bottles were discarded after each treatment time. For each test condition, the liquid fraction was separated by centrifuging the pre-fermented sludge at 2500x *g* for 5 min. Chemical analyses to determine the extent of solubilisation and VFA production where done after filtering the liquid fraction through a 0.45 µm PES Millipore® filter. In the anaerobic pre-fermentation tests, the headspace composition of each serum bottle was analysed just before processing the sample.

2.2.2 Anaerobic digestion experiments

The second step was determination of the methane potential of the solid fraction separated after pre-fermentation; and this was evaluated using the biochemical methane potential (BMP) tests. BMP tests were carried out following the procedure defined by Angelidaki et al. (2009) at mesophilic temperature (37±1 ºC). BMP tests were performed in triplicate in 160 mL serum bottles sealed with rubber septa and aluminium caps. The serum bottles contained inoculum and the amount of substrate required to achieve an initial inoculum to substrate ratio of 2 (VS-basis). Blank assays containing only inoculum were used to correct for the background methane potential of the inoculum. Next, the headspace of each bottle was flushed with 99.9% N_2 for one minute (4 L min^{-1}). Finally, the bottles were placed in an incubator set at 37 °C. Serum bottles were manually mixed by swirling before each sampling event. Accumulated volumetric methane production was calculated from the pressure

increase and methane composition of the headspace at each sampling event. Methane yields are reported at standard conditions (i.e. 0 ºC and 1 bar).

2.3 Analytical methods

Total solids (TS) and volatile solids (VS) were measured according to Standard Method 2540G (APHA, 2012); volatile fatty acids (VFA) and alcohols losses were taken into account to correct the final TS and VS value (Peces et al., 2014). Total chemical oxygen demand (tCOD) and soluble chemical oxygen demand (sCOD) were measured using a Merck COD Spectroquant® test kit (range 0.5-10 g L^{-1}) and a Move 100 colorimeter (Merck, Germany). Individual VFA (acetate, propionate, butyrate, valerate, and caproate) and alcohols (methanol, ethanol, and butanol) were analysed with an Agilent 7890A gas chromatograph equipped with an Agilent DB-FFAP column. Biogas composition (CH_4, CO_2, and H_2) was determined using a Shimadzu GC-2014 gas chromatograph equipped with a HayeSep Q column as for Astals et al. (2015).

2.4 Model implementation and data analysis

The impact of pre-fermentation and separation of soluble products on methane yield was assessed by mathematical analysis of the BMPs. Degradation extent and apparent degradation kinetics were the two targeted parameters to compare the performance of different pre-fermentation conditions. As hydrolysis was considered to be the rate-limiting step during the AD of PS, BMPs were modelled using first order kinetics following Eq. 1 (Jensen et al., 2011).

$$r = f_i \cdot k_{hyd,i} \cdot X_i \cdot C_i \qquad \text{Eq. 1}$$

where r is the methane production rate (L COD-CH_4 day^{-1}), f_i is the substrate biodegradability (-), $k_{hyd,i}$ is the first order hydrolysis rate constant of the substrate (day^{-1}), X_i is the substrate concentration (g VS L^{-1}), and C_i is the measured COD-to-VS ratio of the substrate (COD:VS = 1.40 gCOD gVS^{-1}). To normalise and analyse model outputs, the biodegradability (f_i) was estimated as per Eq. 2.

$$f_i = \frac{B_0}{B_{0,max}} \qquad \text{Eq. 2}$$

where B_0 is the measured methane yield, and $B_{0,max}$ is the maximum theoretical methane yield at standard conditions (350 · COD:VS = 490 mL CH_4 g^{-1} VS).

The model was implemented in Aquasim 2.1d. Parameter estimation and uncertainty analysis were simultaneously estimated, with a 95% confidence limit, as described in Batstone et al. (2009) and Jensen et al. (2011). Parameter uncertainty was estimated based on a two-tailed t-test on parameter standard error around the optimum, and non-linear confidence regions were tested to confirm the linear estimate was representative of true confidence. The objective function used was the sum of squared errors (χ^2), where average data from triplicate experiments were used.

2.5 Data analysis

2.5.1 Solubilisation and acidification yields

The solubilisation yield and the acidification yield were calculated following Eq. 3 and Eq. 4, respectively.

$$\text{solubilisation yield} = \frac{sCOD_f - sCOD_0}{VS_0} \qquad \text{Eq . 3}$$

$$\text{acidification yield} = \frac{VFA_f - VFA_0}{VS_0} \qquad \text{Eq . 4}$$

where $sCOD_f$ and $sCOD_0$ are the soluble COD (g COD L^{-1}) at the end and at the beginning of the pre-fermentation; and VFA_f and VFA_0 are the total VFA concentration expressed in COD equivalents (g COD L^{-1}) at the end and at the beginning of the pre-fermentation; and VS_0 is the initial concentration of volatile solids (kgVS L^{-1}). Solubilisation and acidification yields can be converted to COD-basis by dividing by 1.40 (COD:VS ratio of the PS).

2.5.2 Overall methane yield (B')

The overall methane yield (B') expresses the methane yield of the pre-fermented sludge in terms of the PS initial organic matter content (Eq. 5). B' is used to normalise the PS methane yield by taking into account the organic matter losses occurring during waste processing (Astals et al., 2015).

$$B' = B_0 \cdot (1 - \rho) \qquad \text{Eq. 5}$$

where B' is the overall methane yield (LCH_4 $kg^{-1}VS$), B_0 is the methane yield of the waste after the pre-fermentation (LCH_4 $kg^{-1}VS$), and ρ is the organic matter losses expressed as per unit (gVS_{final} $g^{-1}VS_{initial}$).

3 Results and Discussion

3.1 Extraction of valuable compounds from primary sludge

3.1.1 Organic matter solubilisation

Fig. 1 represents the breakdown of COD during pre-fermentation at all tested conditions. After pre-fermentation at 20 ºC (semi-aerobic and anaerobic) and 37 ºC the soluble COD in the effluent was completely composed of VFA. In contrast, after thermophilic (55 – 70 ºC) pre-fermentation approximately 65% of soluble COD in effluent was undetermined soluble substances (e.g. saccharides, amino acids, and long chain fatty acids). In all scenarios, the solubilisation yield increased with the temperature and the treatment time. At 20 ºC, the sCOD increased gradually up to 8% of the initial tCOD (anaerobic, 72 h), while at 70 ºC the sCOD increased more rapidly to 16% of the initial tCOD (anaerobic, 72 h) (Fig. 1E). These results indicate that when VFA recovery is the main process objective, pre-fermentation should occur at psychrophilic or mesophilic conditions. Nonetheless, pre-fermentation at 20 ºC and at 37 ºC also resulted in 9 and 14% COD losses (anaerobic, 72 h), respectively, due to carbon mineralisation (Table SI - supplementary data). COD mineralisation was not related to methanogenesis or hydrogen production, since these products accounted for less than 1% of the initial COD at 20 ºC (anaerobic) and approximately 1% of the initial COD at 37 ºC after 72 h of pre-fermentation. Therefore, the COD mineralisation was hypothesised to be due the COD consumption from other processes such as sulphate reduction. COD mineralisation at thermophilic conditions was negligible. This factor should be considered when estimating energy recovery in the WWTP, since uncontrolled COD mineralisation during pre-fermentation reduces VFA recovery efficiency as well as the organic matter available for methane production in the subsequent anaerobic digestion step.

3.1.2 VFA distribution

Controlling fermentation products during mixed culture fermentation of complex substrates is a difficult task with process performance depending on several factors such as substrate composition, temperature, pH, retention time, and the microbial community. Despite this, some VFA distribution trends could be observed depending on the pre-fermentation temperature and treatment time (Fig. 2).

Acetate was, regardless the treatment time, the major VFA contributor at 20 ºC (semi-aerobic and anaerobic), 55 ºC and 70 ºC. Among them pre-fermentation at 20 ºC, either semi-aerobically or anaerobically, delivered the richest acetate stream (1.63, and 2.26 $g_{Acetate}$ L^{-1}, respectively). PS pre-fermentation at 37 ºC favoured propionate production (Fig. 2D), with an acetate to propionate ratio of 0.77 (COD-basis) after 72h pre-fermentation. Propionate was also obtained, in lower amounts, after 20 ºC pre-fermentation; whereas 55ºC pre-fermentation led to the accumulation of butyrate and ethanol (Fig. 2E). Similar VFA distribution profiles have been reported by Ahn and Speece (2006) and Ucisik and Henze (2008) when fermenting PS under similar conditions. In terms of net VFA production, the highest acidification yield was reached at 37 ºC (143 $gCOD_{VFA}$ $kg^{-1}VS$) followed by 20 ºC anaerobic (65 $gCOD_{VFA}$ $kg^{-1}VS$) and 20 ºC semi-aerobic (43 $gCOD_{VFA}$ $kg^{-1}VS$). The lowest acidification yields were obtained at thermophilic conditions, with 23 $gCOD_{VFA}$ $kg^{-1}VS$ at 55 ºC, and negligible at 70 ºC (Table 2). Therefore, different pre-treatment conditions would be required depending on the amount of VFA required and the desired profile.

The low acidification yields observed at 55 and 70 ºC could be attributed to the slow development of an anaerobic thermophilic culture during pre-fermentation, since no adapted inoculum was used, and the test conditions relied on the activity of native PS microorganisms. However, higher acidogenic activities have been reported at thermophilic temperatures up to and exceeding 70 ºC (Bolzonella et al., 2007; Ge et al., 2011; Lu et al.,

2008), suggesting that different results might be observed when using microbes acclimatised to this temperature range.

3.2 Extraction of soluble compounds and influence on PS methane yield

Fig. 3 displays the experimental methane production profiles of the solid fraction of pre-fermented PS for all experimental conditions. The results show that the pre-fermentation step under anaerobic conditions neither favoured nor decreased the solid-fraction B_0 with values of approximately 340 $LCH_4\,kg^{-1}VS$ for all anaerobic pre-fermentation temperatures and treatment times (Table 2), showing no statistical differences between themselves and the control (see Fig.SI - supplementary data). However, 20 ºC semi-aerobic conditions significantly increased B_0 compared to the control. Specifically, B_0 after 72h pre-fermentation increased from 336 ± 14 $LCH_4\,kg^{-1}VS$ to 381 ± 14 $LCH_4\,kg^{-1}VS$ (P=0.0091), which represents a methane potential increase of 14%.

Substrate B_0 is a common parameter for assessing the feasibility and expected performance of AD processes. However, this parameter does not reflect the methane losses due to removal and/or mineralisation of organic material during waste processing (e.g. pre-treatment, VFA/product recovery, sulphate reduction) (Astals et al., 2015). Therefore, the overall methane yield (B') was used to evaluate the influence of product extraction and uncontrolled COD losses on the methane production in terms of PS initial organic matter content. As shown in Table 2, anaerobic pre-fermentation decreased sludge B' up to a 21%. Thus, under anaerobic conditions VFA recovery and COD losses, significant at 20 ºC (9%) and 37 ºC (14%), contributed to reduce the methane production in the subsequent AD step. Nevertheless, 20 ºC semi-aerobic pre-fermentation resulted in a minor, but statistically significant increased methane yield even after VFA removal and COD losses (5%) occurring

during the pre-fermentation. This phenomenon coincided with the formation of a white mouldy-like layer on the top of the sludge. The layer was not uniform and consisted of white round patches distributed along the sludge surface, being more spread and prominent after 48h treatment time (especially noticeable at 72h and 96h). Considering the test conditions and the physical appearance of the biomass, it is hypothesised that the organisms were fungi (Fig. 4).

Several groups of fungi have been found in municipal sewage sludge (Fakhru'l-Razi et al., 2002; Kacprzak et al., 2005). These fungi are versatile organic matter consumers, especially at low pH, where bacterial growth is hindered (More et al., 2010). However, in this study the pH of the sludge varied from 5.1 to 4.8, high enough to sustain acidogenic activity. Therefore, in the semi-aerobic conditions, where fungi was observed, fungi may have partially depolymerised complex structures and made available a greater portion of the PS to the fermentative bacteria. Fungi have the capability to degrade cellulose, hemicellulose and polysaccharides by excreting extracellular enzymes (Pointing, 2001) although they are best known for excreting extracellular lignin modifying enzymes that perform lignin degradation. This quality is much less common in anaerobic microorganisms and has prompted the use of fungi as a pre-treatment to enhance the methane potential of lignocellulosic substrates, otherwise difficult to degrade anaerobically (Zheng et al., 2014). In the present study, fungi may have improved the biodegradability of the pre-fermented sludge under semi-aerobic conditions making it more accessible for the subsequent anaerobic microbes; thereby enhancing the overall methane yield (B').

While the specific role of fungi in this study is not completely elucidated, results were repeatable and confirmed the phenomenon (Table 2). However, the magnitude of the effect in the replicated experiment, in terms of acidification yield and methane production, was lower than for the first batch of PS. In either way, results clearly indicate that 20 °C semi-aerobic

pre-fermentation was the only configuration that allowed both VFA recovery and an increase methane production, thereby enhancing overall resource recovery. Results also suggest that a similar phenomenon (i.e. VFA recovery and increased methane recovery) could happen at 37 °C and, to a minor degree, 20 °C anaerobic pre-fermentation if the COD mineralisation mechanisms could be minimised (Table SI - supplementary data).

4 Conclusions

Primary sludge pre-fermentation conditions affected solubilisation yields, VFA profile and methane recovery potential. At 20 and 37 ºC, solubilised COD was mainly VFA with acetate and propionate the major contributors; at 55 and 70 ºC, solubilised COD was mainly other organic compounds. Anaerobic sludge pre-fermentation (37, 55 and 70 ºC) led to higher solubilisation yields but reduced subsequent methane potential by 20%. However, semi-aerobic pre-fermentation at 20 ºC allowed VFA production (43 $gCOD_{VFA}$ $kg^{-1}VS$) and a statistically significant improvement in methane potential. The latter phenomenon was linked to fungi observed growing on the top layer of sludge during pre-fermentation.

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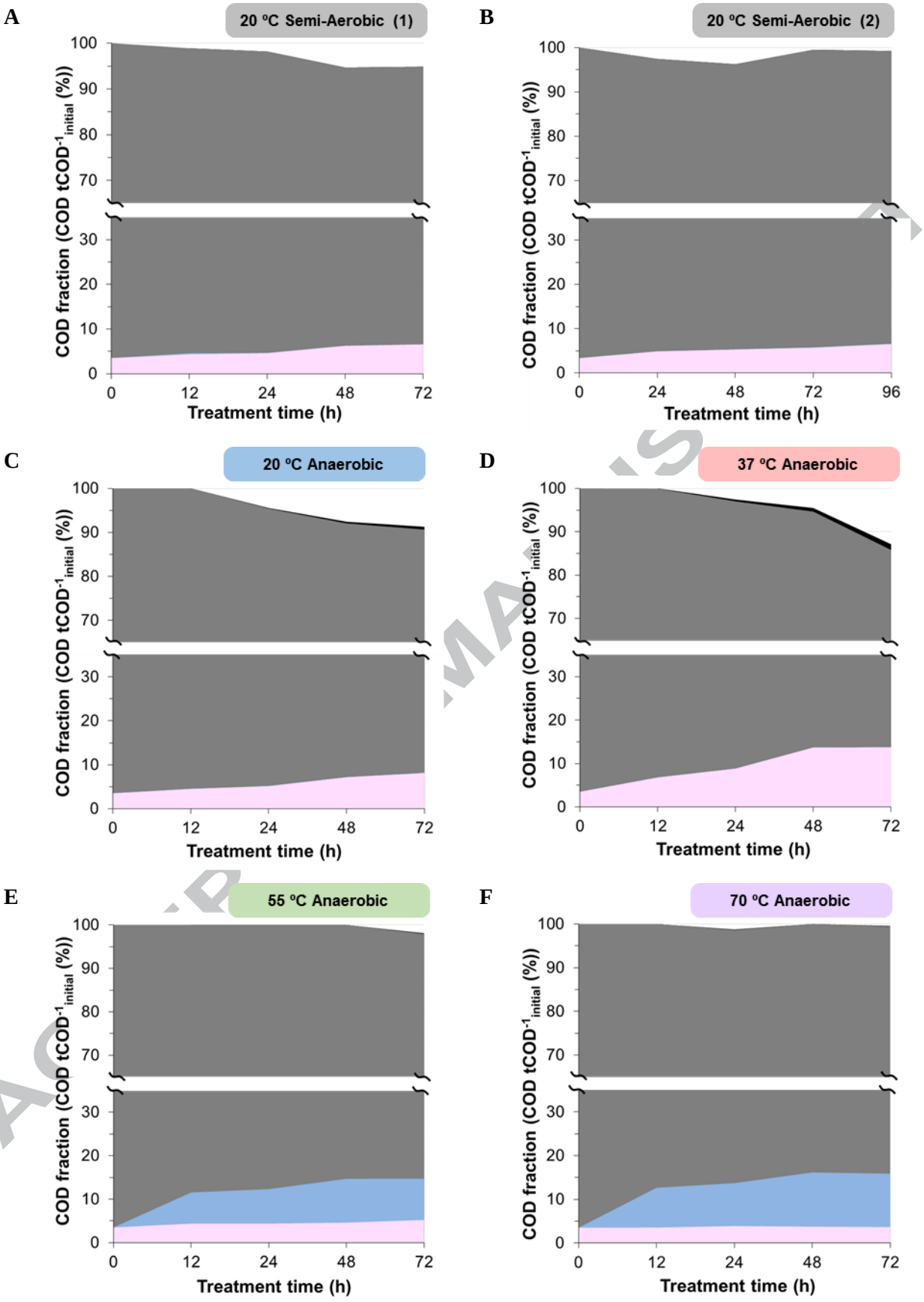

Fig. 1. COD fractionation (in percentage) for each pre-fermentation condition. **(A)** 20 ºC Semi-aerobic, **(B)** 20 ºC Semi-aerobic replicated, **(C)** 20 ºC Anaerobic, **(D)** 37 ºC Anaerobic, **(E)** 55 ºC Anaerobic, and **(F)** 70 ºC Anaerobic. (

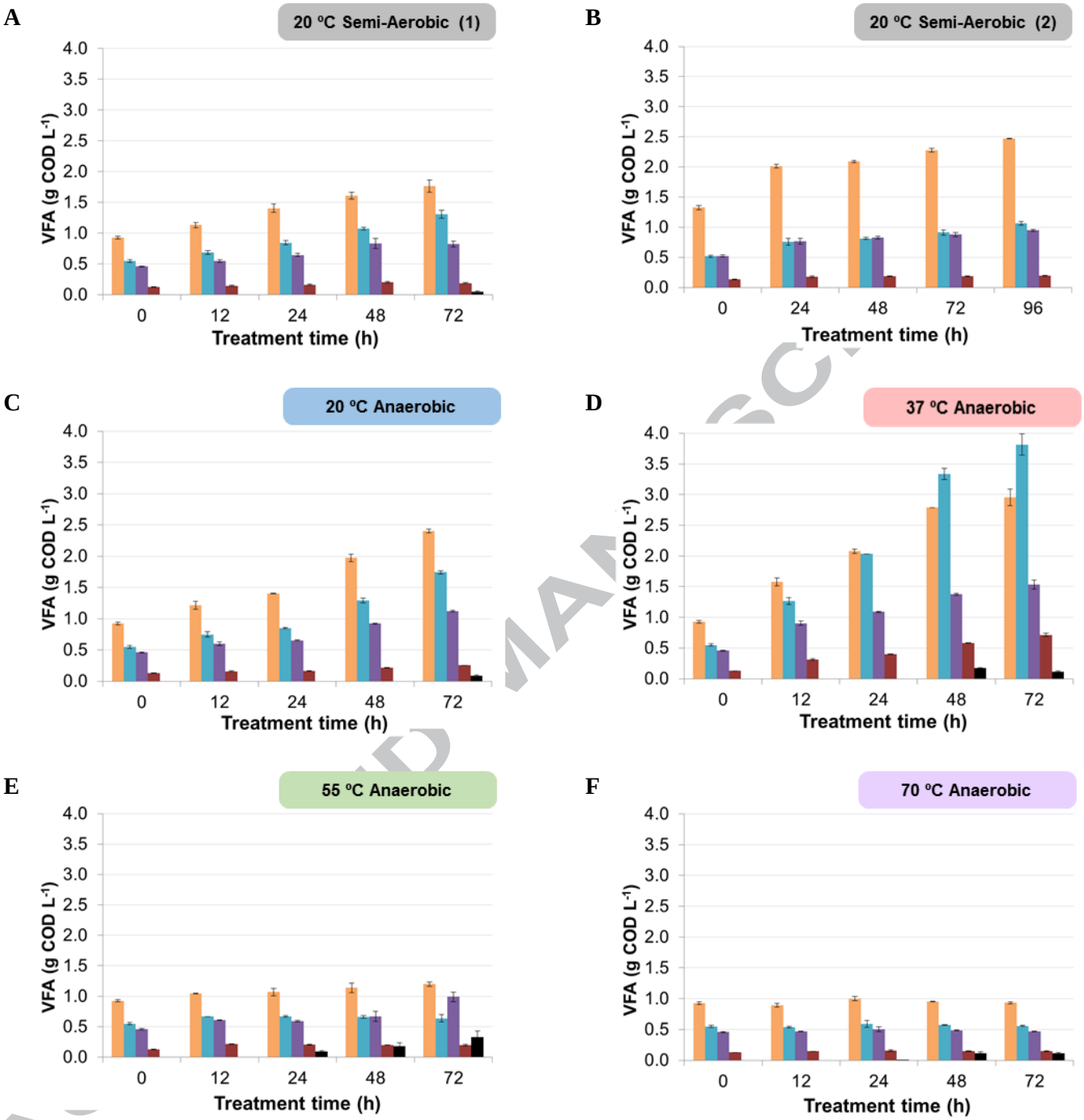

Fig. 2. VFA distribution depending of **(A)** 20 ºC Semi-Aerobic, **(B)** 20 ºC Semi-Aerobic (batch 2), **(C)** 20 ºC Anaerobic, **(D)** 37 ºC Anaerobic, **(E)** 55 ºC Anaerobic, and **(F)** 70 ºC Anaerobic, at the different exposure times applied. (

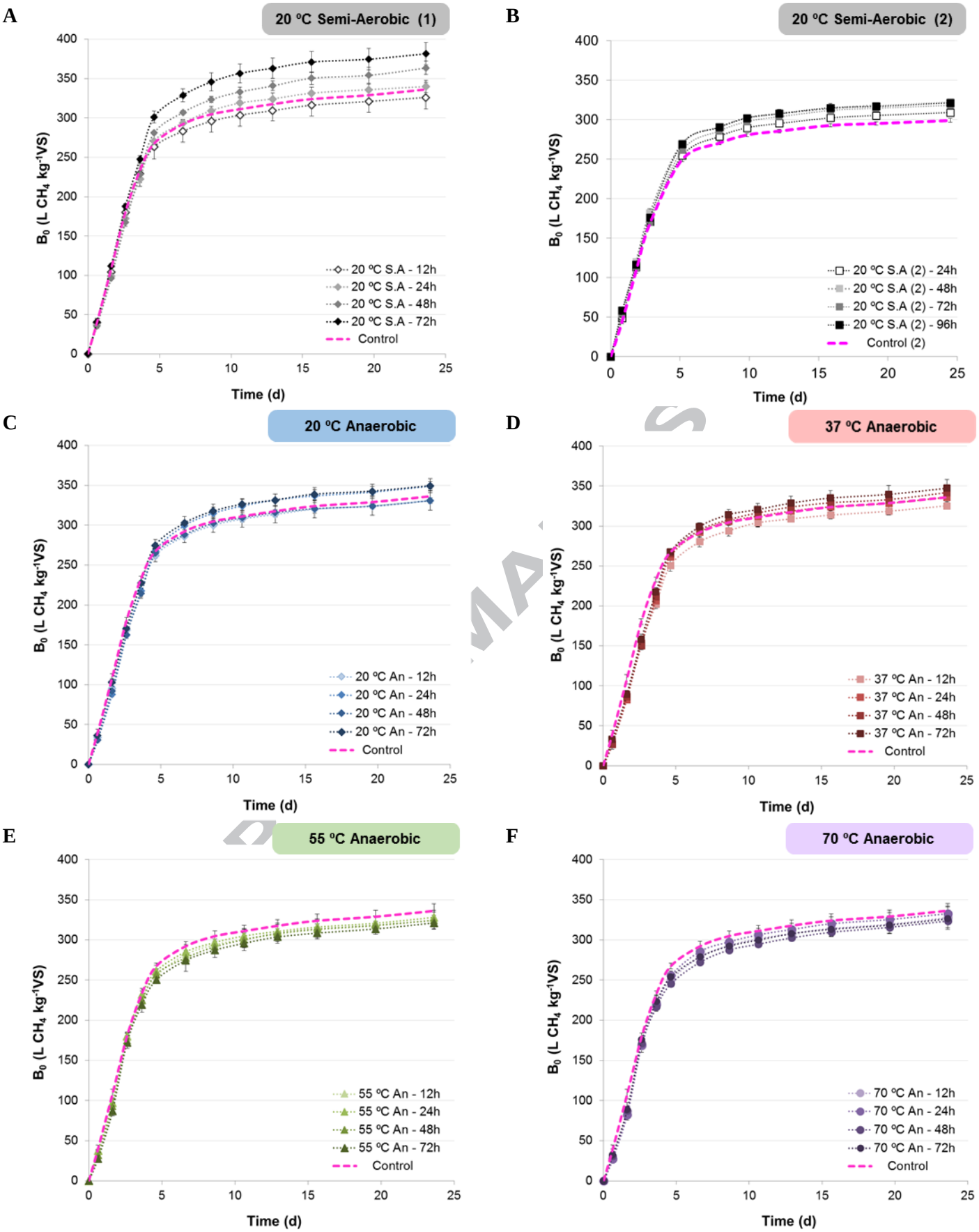

Fig 3. Cumulative specific methane production curves after pre-fermentation at different temperatures, exposure time, and control. **(A)** 20 ºC Semi-Aerobic, **(B)** 20 ºC Semi-Aerobic (batch 2), **(C)** 20 ºC Anaerobic, **(D)** 37 ºC Anaerobic, **(E)** 55 ºC Anaerobic, and **(F)** 70 ºC Anaerobic.

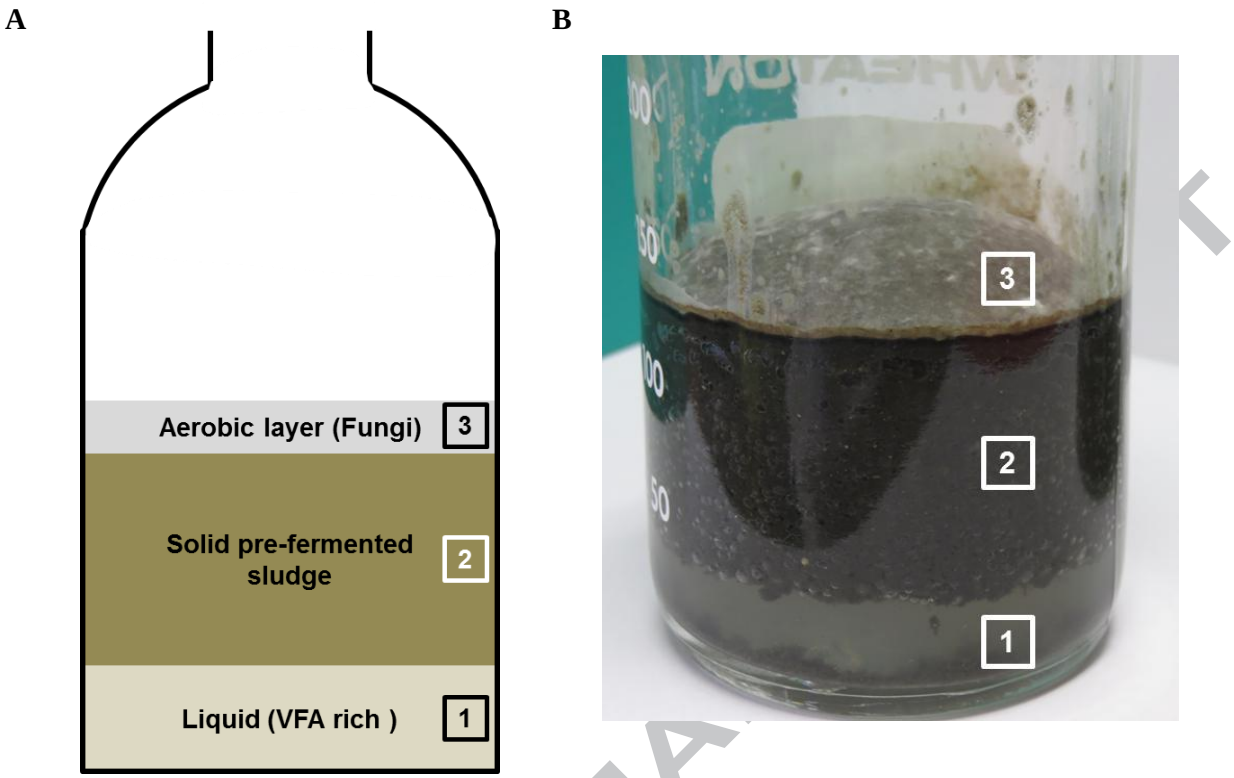

Fig. 4. Primary sludge after 72 h of pre-fermentation (20 ºC Semi-Aerobic). **(A)** Graphic representation. **(B)** Photograph. 1: liquid rich in VFA; 2: residual sludge; and 3: semiaerobic layer colonised by fungi.

Table 1. Characterisation of primary sludge

	Units	Batch 1	Batch 2
TS	gTS L^{-1}	47.9 ± 0.3	52.9 ± 0.2
VS	gVS L^{-1}	41.6 ± 0.2	45.4 ± 0.2
tCOD	gCOD L^{-1}	57.9 ± 0.6	72.1 ± 2.7
sCOD	gCOD L^{-1}	2.0 ± 0.1	2.4 ± 0.1
pH	-	5.1 ± 0.1	4.9 ± 0.1
VFA	gCOD L^{-1}	2.0 ± 0.1	2.4 ± 0.1
Alcohols	gCOD L^{-1}	b.d.l*	b.d.l

* b.d.l., below detection limit (< 1 mg L^{-1})

Table 2. Summary of solubilisation and acidification yields and methane yields for all the pre-fermentation conditions

	Pre-fermentation			Anaerobic digestion			
	Solubilisation yield (g sCOD kg^{-1}VS)	**Acidification yield ($gCOD_{VFA}$ kg^{-1}VS)**	**ρ (gVS_{final} $g^{-1}VS_{in}$)**	**B_0 (LCH_4 kg^{-1}VS)**	**f_i (-)**	**B' (LCH_4 kg^{-1}VS)**	**B' increase (%)**
Raw PS (Batch 1)	-	-	0.03	336 ± 9	0.68 ± 0.02	326 ± 12	-
20 ºC Semi-aerobic							
12 h	14.8 ± 0.3	11.0 ± 2.5	0.06	326 ± 15	0.66 ± 0.02	305 ± 15	-6
24 h	15.6 ± 2.0	15.6 ± 3.2	0.05	340 ± 8	0.69 ± 0.02	323 ± 13	-1
48 h	38.3 ± 1.8	38.3 ± 1.0	0.06	364 ± 9	0.73 ± 0.02	340 ± 10	4
72 h	42.6 ± 1.2	42.6 ± 5.3	0.11	382 ± 14	0.78 ± 0.02	338 ± 14	4
20 ºC Anaerobic							
12 h	14.0 ± 0.6	14.0 ± 3.5	0.08	331 ± 12	0.67 ± 0.02	306 ± 12	-6
24 h	23.1 ± 4.0	23.1 ± 0.7	0.10	349 ± 6	0.71 ± 0.03	315 ± 9	-3
48 h	51.0 ± 2.6	51.0 ± 0.1	0.13	331 ± 4	0.68 ± 0.02	298 ± 10	-11
72 h	64.4 ± 1.5	64.4 ± 1.8	0.14	349 ± 9	0.71 ± 0.02	302 ± 14	-7
37 ºC Anaerobic							
12 h	46.1 ± 1.1	46.1 ± 4.1	0.13	325 ± 5	0.66 ± 0.02	283 ± 8	-13
24 h	74.2 ± 2.0	74.2 ± 1.1	0.15	336 ± 14	0.69 ± 0.03	285 ± 15	-12
48 h	141.9 ± 4.3	141.9 ± 2.6	0.23	342 ± 9	0.70 ± 0.03	263 ± 15	-19
72 h	142.7 ± 4.8	142.7 ± 10.5	0.25	347 ± 11	0.71 ± 0.02	259 ± 10	-21
55 ºC Anaerobic							
12 h	112.0 ± 2.5	11.5 ± 0.1	0.15	325 ± 6	0.65 ± 0.02	276 ± 8	-15
24 h	122.8 ± 2.4	11.4 ± 1.1	0.17	328 ± 9	0.66 ± 0.02	271 ± 9	-17
48 h	155.8 ± 3.2	14.6 ± 0.5	0.19	324 ± 10	0.66 ± 0.02	263 ± 10	-19
72 h	156.2 ± 2.7	23.3 ± 0.5	0.16	321 ± 7	0.65 ± 0.02	271 ± 7	-17
70 ºC Anaerobic							
12 h	127.3 ± 0.4	0.0 ± 0.0	0.19	328 ± 13	0.66 ± 0.02	266 ± 11	-18
24 h	142.1 ± 0.5	4.7 ± 0.7	0.21	333 ± 9	0.67 ± 0.02	264 ± 11	-19
48 h	176.3 ± 2.9	2.4 ± 0.1	0.18	323 ± 11	0.65 ± 0.02	264 ± 10	-19
72 h	172.2 ± 2.6	1.2 ± 0.2	0.18	326 ± 5	0.66 ± 0.02	266 ± 5	-18
Raw PS (Batch 2)	-	-	0.04	299 ± 3	0.54 ± 0.02	287 ± 7	-
20 ºC Semi-Aerobic							
24 h	24.5 ± 0.8	23.4 ± 0.9	0.04	309 ± 4	0.55 ± 0.01	298 ± 5	4
48 h	31.1 ± 0.2	31.4 ± 1.3	0.05	323 ± 3	0.58 ± 0.02	307 ± 7	6
72 h	38.1 ± 0.9	37.3 ± 1.2	0.07	318 ± 4	0.57 ± 0.01	296 ± 10	3
96 h	56.1 ± 0.1	48.3 ± 1.3	0.08	321 ± 4	0.58 ± 0.02	295 ± 12	2

Highlights

- Temperature of primary sludge fermentation affected acidification yield and profile
- Fermentation at 20 & 35°C led to VFA, while 55 & 70°C led to other soluble material
- VFA removal after 37, 55 & 70ºC fermentation lowered 20% sludge methane potential
- Semi-aerobic conditions at 20°C improved methane yield even after VFA removal
- Fungi colonised the sludge top layer under 20°C semi-aerobic conditions