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Particle size, inoculum-to-substrate ratio and nutrient media effects on biomethane yield from food waste

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Abstract

This study investigates the effects of particle size reduction at different inoculum-to-substrate ratios and nutrient media supplementation on the assessment of biomethane production from food waste, under batch mesophilic conditions. Two different food waste samples were used and the best method for testing biomethane potential was chosen based on their characterisation and methane yields. Results obtained indicate that Inoculum-to-substrate ratios of 3:1 and 4:1 helped to stabilise test reactors with smaller particle sizes of 1 mm and 2 mm, respectively. Consequently, an overall biomethane yield increase of 38% was reported (i.e., from 393 $NmLCH_4$ gVS^{-1}_{added} to 543 $NmLCH_4$ gVS^{-1}_{added}). This could potentially imply a better assessment of energy outputs from anaerobic digestion of food waste (i.e., 43.5% higher energy output as electricity from biogas, using commercial scale Combined Heat and Power (CHP) units). Although nutrient media supplementation did not enhance methane yield from optimum inoculum-to-substrate ratio (3:1) and particle size (1 mm), it was found that its application helped to stabilise food waste digestion by avoiding volatile fatty acids accumulation and high propionic-to-acetic acid ratio, consequently, improving the overall test kinetics with 91% lag time reduction from 5.6 to 0.5 days. This work supports the importance of key variables to consider during biomethane potential tests used for assessing methane yields from food waste samples, which in return can potentially increase the throughput of anaerobic digestion system processing food waste, to further increase the overall energy output.

Keywords: Anaerobic digestion; Food waste; Methane yield; Nutrient media supplementation; Particle size reduction

1. Introduction

In the United Kingdom (UK) around 10 million tonnes/year of food and drink is wasted in the post-farm food chain; with the highest proportion being produced by households (7 million tonnes), followed by the manufacturing sector (1.7 million tonnes). However, 60% of this waste could have been avoided, being good enough to have been consumed at some point prior to its disposal (WRAP, 2015). Important drivers such as the increasing public awareness and concerns regarding environmental quality degradation, together with the rapidly rising costs related to energy supply and waste disposal, have promoted the development of food waste to energy practices worldwide (Zhang et al., 2007). A commonly used method throughout Europe is Anaerobic Digestion (AD), since it can treat and stabilise organic matter, as well as producing renewable energy in the form of biomethane (Pullen, 2015).

AD in the United Kingdom is already well established. There are currently over 540 operational AD plants in the UK (REA, 2017), most of them operating in commercial scale and processing different types of organic wastes including: food waste (FW), sewage sludge, manure, slurries, crop residues and purpose-grown crops, and of this total, over 50 anaerobic digesters treat food waste (WRAP, 2012). The AD process consists of four steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis (Appels *et al*., 2011). Amongst the successive reactions, hydrolysis and sometimes acidogenesis are considered to be the rate limiting steps, affecting the mass transfers and substrate availability within the system (Zhang *et al*., 2014). To enhance the organic matter solubilisation and avoid any impact from the rate-limiting steps, several pre-treatments methods have been applied to food waste prior to anaerobic digestion process including: chemical (Ma *et al*., 2011), biological (Gonzales *et al*., 2005), and physical strategies (Shahriari *et al*., 2013).

As part of the physical pre-treatments for FW there is the mechanical gridding, which allows Particle Size (PS) reduction. Smaller particles ultimately increase biodegradability by expanding the surface area and subsequently, food availability to the microbial community, thus improving methane production (Mshandete *et al*., 2006, Izumi *et al*., 2010). In agreement, Kim *et al*., (2000) reported that by reducing food waste PS from 2.14 to 1.02 mm the maximum substrate utilization doubled, thus improving process performance. Meanwhile, in some cases PS reduction can have a detrimental effect as suggested by Izumi *et al*., (2010), stating a negative relationship between excessive PS reduction and methane production.

Moreover, methane production from food waste can also be enhanced using different inoculum-to-substrate ratio (ISR). Neves *et al*., (2004) assessed the biomethane potential of kitchen waste by testing a range of ISR (2, 1, 0.74 and 0.43), along with two inoculum types (granular and suspended). The authors concluded that acidification was successfully prevented over the tested ISR range when granular inoculum was used. Suspended sludge on the other hand, only avoided acidification at the highest ISR. Similarly, Lopes *et al*., (2004) applied a bovine fluid inoculum at ISR 0.17, 0.11, and 0.05 to assay the biostabilisation of the organic fraction of municipal solid waste, revealing a straight-forward relation between higher amounts of inoculum and process performance improvement. Although previous studies have investigated the individual effect of PS and ISR on biomethane yield, a further combination of PS with ISR towards biomethane improvement from AD, as at the time of conducting this study, have not yet been reported in the literature.

Despite the various methods to improve biodegradability and biomethane production from FW, it has been shown that digestion of this substrate alone has often proven difficult and rarely reported as successful (Tampio *et al*., 2014), especially in a single-stage process. The main difficulty is related to the fact that most food waste are trace-element deficient substrates. Thus, important nutrients to the AD biochemical pathways, especially to the methanogenesis step such as Co, Ni, W, Se and Mo are often found in very low concentrations or even absent (Facchin *et al*. 2013). However, with appropriate nutrient supplementation the AD process of FW becomes more resistant to environmental changes, hence more efficient (Banks *et al*., 2012; Climenhaga and Banks, 2008; El-Mashad *et al*., 2008; Lee *et al*., 2009).

Therefore, the principal aims of this paper were as follows: a) determine whether the combination of PS reduction and ISR could enhance process stability and the assessment of biomethane yield from food waste anaerobic digestion, and b) to investigate if nutrient media supplementation can enhance even further the biomethane yield of food waste under the optimum PS and ISR, using batch biochemical methane potential (BMP) assays at mesophilic temperatures.

2. Material and methods

2.1 Food waste collection, processing and particle size characterization

Food waste was collected from the Leeds University Refectory, Leeds, United Kingdom, on two different occasions. The first collection occurred during a single visit to the establishment. Due to its composition reflecting mainly raw, uncooked ingredients from the kitchen area of the refectory, this sample was denominated Kitchen Waste (KW). The second collection happened over five consecutive days and composite samples consisted of both plate waste (from the eating area) and kitchen wastes, hence denominated as Composite Food Waste (CFW) samples (Table II). The two sampling streams were conducted to understand the effect of particle size, inoculum-to-substrate ratio and nutrient media on the effective biomethane potential of different food waste streams likely to be produced at household level (i.e., uncooked food waste and food waste), using food waste from the refectory as a proxy.

Samples were collected on the same day they were discarded, as suggested by Zhang *et al.*, (2007), thus avoiding dealing with putrescible waste and consequently, underestimating Total Solids (TS) and/or Volatile Solids (VS) results. The collected waste was manually sorted for any unwanted impurities such as glass, paper, cardboard, plastic and bones. Sorted food waste substrate was thoroughly mixed, chopped and ground with a mincer. To allow further substrate size reduction and better homogenisation, the sample was blended with a food liquidizer. During this process, no water was added so the moisture content would not be affected. After the homogenisation and particle reduction step, the PS for the raw food waste was characterised by sieving a known amount of sample through a series of sieves with aperture between 1 and 10 mm and comparing the recovered solids to the reject to achieve a solids recovery of not less than 95%. Below an aperture of 5 mm the solids recovery was less than 95%, hence, the raw homogenised food waste PS was characterised as ≤5 mm. Subsequently, food waste samples with a PS of 1 mm and 2 mm were achieved by sieving the raw homogenised food waste sample through the respective sieve. Due to the dense and paste nature of the sample, it was not possible to allow it to drain freely through the sieves, therefore, manual pressure was applied during the sieving process using a flat metal bar. Hence, the first food waste PS was the undersize of the processed sample from 1 mm sieve, the second PS was the undersize of the processed sample from a 2 mm sieve and the last was the raw homogenised sample after processing with PS ≤5 mm; having 95% solids recovery from a 5 mm sieve.

To generate representative sub-samples, the food waste sample for each PS group was individually mixed and divided into four samples. Subsequently, smaller samples of 500 g were weighed into refrigerator bags, labelled and stored at -20 °C until required for the experiments; one bag from each sample was however stored at 4 °C to carry out the characterisation. Frozen samples used for the experiments were thawed at 4 °C prior to BMP experiments; such that no heat was added to defrost the samples.

2.2 Inoculum

The inoculum used in this study was obtained from a mesophilic anaerobic digester, treating sewage sludge at Esholt Wastewater Treatment Plant in Yorkshire, UK. Before each experimental set-up the inoculum was passed through a 1 mm sieve to remove any large particles or grit and then incubated at 37 °C. Acclimation of the inoculum to food waste was done over a 30 days period, by adding 3 g_{FW} $L^{-1}_{inoculum}$ once every two weeks, equivalent to 0.2 gVS_{FW} L^{-1} day^{-1}. Since the experiments were carried out in distinct timeline, the adapted inoculum (henceforth referred to as inoculum) was characterised regarding its main physical-chemical properties two days before each BMP set-up.

2.3 Experimental design

2.3.1 Anaerobic biodegradability (BMP) tests

This step consisted of two sets of experiments. Experiment 1 tested the effect of combining different PS and ISR on the biomethane yield of KW. Once the optimal conditions of ISR and PS for improved biomethane yield were established with KW, the biomethane yield at the same conditions were conducted with CFW in comparison with KW. Considering that KW and CFW samples had similar biomethane yields, Experiment 2 was conducted to test the effect of nutrient media supplementation to further improve the biomethane yield using CFW samples only. The decision of applying nutrient media supplementation on CFW was based on the results from food waste characterisation – having higher theoretical methane potential (TMP), but less metal content than KW. BMP trials were conducted in batches using 500 ml Duran bottles, with 400 ml working volume, under mesophilic conditions (37 °C). The temperature was maintained by means of a water bath as part of the automatic methane potential test system (AMPTS II) by Bioprocess Control as described by Browne *et al*., (2013). To determine the biomethane originating from the inoculum, blank samples were prepared for each set of experiment, containing only inoculum and distilled water. A 3^2 factorial design was employed for Experiment 1; that is three levels of food waste PS and three levels of ISR (Table I). All BMP assays were conducted in triplicates.

Table I. Experimental set-up for Experiment 1.

Particle size, PS (mm)	Inoculum to Substrate Ratio, ISR	Volatile Solids (VS) content (g/Reactor)
1	2	8.10
1	3	11.38
1	4	6.75
2	2	9.05
2	3	8.04
2	4	7.54
5	2	5.72
5	3	5.08
5	4	4.76

2.3.1.1 Experiment 1: Applying different Food Waste Particle Size and Inoculum-to-Substrate Ratios

The food waste samples were blended with a Nutribullet homogeniser and characterised as ≤5 mm; having >95% recovery of the food waste from a 5 mm screen. They were then sieved through 1 mm and 2 mm screens to obtain the respective PS, as such the three PS (≤1 mm, ≤2 mm and ≤5 mm); hereafter denoted as 1 mm, 2 mm and 5 mm, were added to each reactor as a substrate, at different concentrations, depending on the ISR used. These sizes were chosen because smaller PS below 1 mm could encourage high volatile fatty acids (VFAs) concentration, due to enhanced fermentation (Izumi *et al*., 2010), while above 5 mm lower biogas yield could be obtained, due to poor substrate degradation. Three ISR were tested; 2:1, 3:1 and 4:1 based on VS content.

When assembling the reactors, a fixed volume of 300 ml of inoculum was used for all assays and the VS concentration in this amount of inoculum was calculated. For each ISR, the required amount of food waste was determined. Hence, the calculated FW amount was added to 300 ml of inoculum and made up to 1 litre with distilled water. Bulk samples were prepared with constant manual mixing and divided into aliquots of 500 ml; out of which 400 ml was used for the BMP analysis, while the 100 ml samples remaining were used to conduct the experimental analysis for day 0 (when the reactors were assembled). The reactors were continuously flushed with pure N_2 gas for 1 minute to ensure anaerobic conditions of the reactors and capped tightly with rubber stoppers.

2.3.1.2 Experiment 2: Applying nutrient media to improve methane yield

The CFW was used in Experiment 2 and tested at ISR of 3:1. Although, the KW and CFW had similar biomethane yields at optimum conditions of PS and ISR, the lower C/N ratio and nutrient content, as well as the higher TMP of the CFW, suggested that its supplementation with macro- and micro-nutrient media could further enhance methane production. The nutrient media composition and preparation was based on previous works (Adewale, 2014, Angelidaki *et al*., 2009, Owen *et al.,* 1979 and Kim *et al*., 2003). Four stock solutions A, B and C and D were used to prepare the final nutrient media and the concentration of chemicals in each solution is given below in g L^{-1} in distilled water.

Solution A: NH_4CL (0.53), KH_2PO_4 (0.27), K_2HPO_4 (0.35), $CaCl_2.2H2O$ (0.075), $MgCl_2.6H_20$ (0.10), $FeCl_2.4H_20$ (0.02), $MnCl_2.4H_20$ (0.05), H_3BO_4 (0.05), $ZnCl_2$ (0.05), $CuSO_4$ (0.03), $Na_2MoO_4.2H_2O$, (0.01), $CoCl_2{\cdot}6H_2O$ (0.50), $NiCl_2.6H_2O$ (0.05).

Solution B: Biotin (0.002), Folic Acid (0.002), Riboflavin (0.005), Thiamine (0.005), Nicotinic Acid (0.005), Cobalamin (0.0001), p-aminobenzoic acid (0.005).

Solution C: 500 g of $Na_2S.9H_2O$ in 1 L of distilled water.

Solution D: 0.5 g of Resazurin in 1L of distilled water as an oxidation-reduction indicator.

Solution A was used as a base solution and autoclaved for 15 minutes at 121°C and 103.4 KPa. Then the other solutions were added to it in the following volumes: 10 ml of solution B; 1 ml of solution C and 1 ml of solution D. Finally, the pH was corrected to 7.0 ± 0.2 by gradually adding $NaHCO_3$; up to a maximum of 1.20 g. When assembling the reactors, 15 g of VS of inoculum was used and the required amount of food waste (in g of VS) was established by dividing it by the respective ISR (3:1). The volume of media used in the reactor was determined by deducting the inoculum and food waste volumes from the 400 ml reactor working volume. No water was used in the reactors with nutrient media, thus possibly avoiding important nutrients becoming a limiting factor on the system. The media was transferred to each reactor, followed by the inoculum and food waste. A Resazurim solution was added to indicate the presence of oxygen inside the reactors. During the media inoculation, the bottles were continuously flushed with pure N_2 gas to ensure anaerobic conditions of the reactors and capped tightly with rubber stoppers.

2.3.2 BMP test monitoring

Liquid samples were analysed on day 0 and then on day 4 (except for Experiment 2 where samples were also analysed on day 7). After this period, sampling was carried out once a week, until the last day of digestion; when the digestate was also characterised. All analytical monitoring during the BMP test was conducted in duplicates.

Daily methane production from each reactor was automatically measured and converted to Standard Temperature and Pressure (STP) conditions (1 atm and 0 °C) by the AMPTS II system. Methane yield was calculated based on the amount of VS added as described in the AMPTS II manual. The total digestion period was 28 days, or when the daily methane production was less than 1% of the total cumulative methane produced by the reactor since the beginning of the experiment – Nielfa *et al.* (2015).

2.3.3 Analytical methods

Standard analytical methods used for the examination of wastewaters and sludge were employed (APHA, 2005) to characterise liquid samples, including the following parameters: total solids - TS (Method 2540 B), volatile solids - VS (2540 E) and chemical oxygen demand - COD (5220 C). The pH of all reactors was measured using a pH meter (HACH, 40d). Elemental carbon, hydrogen, nitrogen and sulphur (CHNS) were measured using Thermo Scientific FLASH2000 Organic Elemental Analyser. Samples were first dried at 40 °C for two days and ground to a powder using a mortar and pestle.

Protein content was performed by determining the nitrogen using the Kjeldahl method, and the lipid content by the Soxhlet extraction method at 40 – 60 °C, using petroleum Spirit as solvent (Nielsen, 2010). Carbohydrate values were obtained by differential method; deducting lipid, protein, ash and moisture content from the total weight of the samples. Volatile Fatty Acids (acetic; propionic; i-butyric, butyric, valeric and i-valeric acid) were measured using a Gas Chromatographer - GC (Agilent Technologies, 7890A) equipped with a flame ionization detector (FID), an auto-sampler and a DB-FFAP column (length 30m, diameter 0.32 mm and film thickness 0.5 µm), and using Helium as a carrier gas. The operating conditions of the GC-FID detector were: 150 ˚C inlet temperature and 200 ˚C FID temperature. Liquid samples were adjusted to pH 2.0 using phosphoric acid and allowed to rest for 30 minutes and then centrifuged at 14,000 RPM (16,000 x *g*) for 5 min, using a Technico Maxi Microcentrifuge. After centrifuging, the supernatant was filtered through a 0.2 µm filter and the liquid analysed for VFAs. The GC was calibrated with SUPELCO Volatile Acid Standard Mix, which includes acetic-, propionic-, iso-butyric-, butyric-, iso-valeric-, valeric-, iso-caproic-, caproic- and heptanoic- acids. The concentration of the various trace elements and metals were determined by AOAC Method 2015.01, for heavy metals in food, by Inductively Coupled Plasma – Mass Spectrometry (ICP-MS), using microwave-assisted acid digestion (nitric acid and hydrogen peroxide) (AOAC, 2013).

2.3.4 Data processing and statistical analysis

The estimation of the theoretical methane potential (TMP) was calculated based on the Buswell equation (Buswell, 1952). A kinetic analysis of the methane production and soluble COD degradation was conducted. The modified Gompertz (MGompertz) growth model (Equation 1) was used to fit the methane production curves, according to Zwietering *et al.*, (1990), to estimate the lag phase and maximum specific methane production rate for each assay, using Origin-Pro® 2018 graphical and statistics software.

$$y = Aexp\left\{-exp\left[\frac{\mu_m \cdot e}{A}\,(\lambda - t) + 1\right]\right\} \qquad \text{Eq. 1}$$

where; y= *Cumulative methane yield* ($mLCH_4$ gVS^{-1}_{added}), A=*Maximum methane yield* ($mLCH_4$ gVS^{-1}_{added}) *at time* t, μm=*Maximum specific methane yield per day* ($mLCH_4$ (gVS^{-1}_{added} Day^{-1})), λ=*Lag phase* (*Days*), e = *exp* (1).

Coupled with the kinetic fitting, a full factorial design of experiment (DOE) was constructed using Minitab18 statistical software to analyse the variance between the BMP data from Experiment 1, using a 2 factor and 3 levels (3^2) factorial design. A surface regression analysis was also conducted with the structured DOE to further examine the effect of intermediate PS (3 and 4) effect on the biomethane yield at a confidence level (α) of 0.05.

3. Results and discussion

The composition of both KW and CFW are described in Table II. CFW samples had a broader composition than KW, possibly because of a longer collection period compared to KW. The physical and biochemical characteristics of both samples are shown in Table III.

Table II. Composition of food waste samples.

Sample	**Component**
Kitchen Waste (KW)	Pineapple, water melon, casaba melon, strawberry, red, green and yellow pepper, carrot, cucumber, lettuce, tomato, white rice, potatoes (harsh brown) and white buns.
Composite Food Waste (CFW)	Tomato, chickpeas, cucumber, green peas, mushroom, carrot, fried and cooked potatoes, potatoes peels, rocket leaves, onions, broccoli, green beans, corn, red pepper, okra, bread, pizza, spaghetti, Yorkshire pudding, rice, fried and boiled eggs, bacon, beef, fish chicken, sausages, minced meat, baked beans and butter.

Table III. Physical and Biochemical Characteristics of food waste samples and comparison with published literature.*

	Present work		**References**				
Parameter/Sample	**Average Value (standard deviation)**		**Vavouraki *et al.* (2013)**	**Zhang *et al.* (2007)**	**Zhang *et al.* (2011)**	**Zhang *et al.* (2013)**	**Quiang *et al.* (2013)**
	KW	**CFW**	**Kitchen Waste**	**Food Waste**	**Food Waste**	**Food Waste**	**Food Waste**
Moisture Content %	78.58 (0.25)	68.11 (0.30)	81.5(0.66)	-	-	-	-
Total Solids (TS), mg/kg (wet base = w.b.)	21.4 (2.52)	31.9 (3.01)	18.5(0.71)	30.90(0.07)	18.1(0.6)	23.1(0.3)	14.3 (1.75)
Volatile Solids (VS), mg/kg (w.b.)	20.5 (1.36)	29.6 (4.05)	_	26.35(0.14)	17.1 (0.6)	21.0(0.3)	13.1 (1.71)
VS/TS % (dry base = d.b.)	95.58	92.91	94.1 (0.35)	85.30 (0.65)	0.94(0.01)	90.9(0.2)	-
C %TS	50.87 (0.07)	53.06 (0.37)	-	46.78(1.15)	46.67	56.3(1.1)	47.4(0.01)
H %TS	7.21 (0.14)	7.79 (0.10)	-	-	-	-	6.65(0.28)
N %TS	2.96 (0.03)	4.85 (0.07)	-	3.16(0.22)	3.54	2.3(0.3)	1.90(0.09)
O %TS	38.83 (0.24)	34.18 (0.51)	-	-	-	-	43.7(0.28)
S %TS	0.13 (0.01)	0.13 (0.03)	-	-	-	-	0.41(0.06)
C/N	17.19	10.95	-	14.80	13.2	24.5(1.1)	24.94
Lipid % TS	24.25 (0.44)	27.62 (1.36)	14.0(0.51)	-	23.3(0.45)	-	-
Protein % TS	14.33 (0.68)	24.31 (1.00)	16.9(0.69)	-	-	-	-
Carbohydrate % TS	57.52 (0.48)	42.75 (1.97)	24.0 (1.06)	-	61.9	-	-
Calcium (Ca), mg/kg TS	154.2 (3.8)	227.3 (20.4)	-	-	-	-	-
Cobalt (Co), μg/kg TS	3.6 (1.1)	2.8 (0.5)	-	-	-	-	-
Cooper (Cu), mg/kg TS	1.7 (0.2)	1.3 (0.1)	-	-	-	-	-
Chromium (Cr), mg/kg TS	N.D.**	N.D.	-	-	-	-	-
Iron (Fe), mg/kg TS	3.6 (0.4)	4.3 (0.6)	-	-	-	-	-
Nickel (Ni), μg/kg TS	219.1 (58.8)	156.9 (28.1)	-	-	-	-	-
Magnesium (Mg), mg/kg TS	42.8 (2.2)	40.5 (1.1)	-	-	-	-	-
Manganese (Mn), mg/kg TS	1.1 (0.04)	0.6 (0.08)	-	-	-	-	-
Molybdenum (Mo), μg/kg TS	24.6 (4.0)	33.8 (3.5)	-	-	-	-	-
Selenium (Se), μg/kg TS	n.d	391.2 (103.2)	-	-	-	-	-
Potassium (K), mg/kg TS	586.1 (11.5)	773.5 (22.0)	-	-	-	-	-
Tungsten (W), μg/kg TS	5.9 (1.9)	4.5 (0.9)	-	-	-	-	-
Zinc (Zn), mg/kg TS	2.1 (0.5)	4.9 (0.8)	-	-	-	-	-
Total Chemical Oxygen Demand (TCOD), g/L	264.55	327.46 (22.13)	-	-	-	-	-
Total VFAs, mg/L	412.49(25.82)	746.82 (2.65)	-	-	-	-	-
pH	4.20	4.85	-	-		-	-

*Data reported as mean values with standard deviation are in brackets, when available.

** N.D. = Not Detectable

Regarding the composition of the substrate, both samples had a high VS/TS ratio; 95.58 and 92.91% for the KW and CFW respectively, indicating that most components of the wastes are organic matter susceptible of biodegradation, thus its viability as a feedstock for biogas production via anaerobic digestion. Food waste is a substrate known for having low pH ranges. The results found in this study were in consonant with others FW studies, which found a pH range between 4.0 and 5.2 (Browne and Murphy, 2013; Elbeshbishy *et al.*, 2012; De Vrieze et al., 2013; Defra, 2010; Paritosh et al., 2017; Wang et al., 2014; Zhang et al., 2015).

Nevertheless, CFW contained higher concentrations of lipids (27.62%) compared to other food waste samples, including KW, hence, suggesting a likely higher biomethane potential (Zhang et al., 2014). However, the C/N ratio at 5 mm PS (10.95 – 17.19) was lower than the recommended value range of 20 – 30 (Puyuelo *et al.*, 2011). An optimum C/N is required for bacteria to allow their growth and maintain a stable environment, as well as being an important indicator of potential ammonium/ammonia toxicity and inhibition. The significantly lower C/N ratio of the CFW sample (10.95) could hinder the AD process, by decreasing the COD (chemical oxygen demand) removal and VS destruction rates, thus negatively affecting the reactor performance and further methane production (Musa *et al.*, 2014).

The TS content in the KW and CFW were mainly constituted of carbohydrates at 57.52 and 42.75%, followed by lipids at 24.25 and 27.62%, respectively. Protein content was significantly higher in the CFW sample, than the KW sample (1.7 times greater) and other reported elsewhere (1.4 times greater – Table III). This implies the CFW has a higher potential for high ammonia loads and related toxicity.

Based on the inorganic composition of the wastes here studied, the KW sample contained higher concentrations of trace elements compared to the CFW, except for Selenium, which was absent in the former. Overall, based on different waste compositions published in the literature, it is possible to corroborate the representativeness of both samples used in this study, and their suitability for anaerobic biodegradability.

3.1 Experiment 1: Influence of Particle Size and Inoculum-to-Substrate ratio

3.1.1 Influence of Particle Size reduction on food waste elemental characteristics

Mechanical pre-treatment, which mainly involves size reduction, is widely employed in anaerobic digestion, with reported increase in methane yield, especially due to enhanced hydrolysis (Zhang *et al.*, 2014). The reduction in PS and subsequent sample preparation of the 2 mm and 1 mm KW samples resulted in a change in TS from 214.2 g/kg at 5 mm to 209.0 g/kg and 205.9 g/kg at 2 mm and 1 mm respectively. The VS content also slightly changed from 205 g/kg at 5 mm to 200 g/kg at 2 mm and 197 g/kg at 1mm. These negligible changes in TS and VS contents due to sample preparation (larger, heavier samples could have been rejected during sieving) may have impacted on the elemental characteristics of the samples.

Reducing the PS in this study resulted in an increase in C/N ratio. The C/N ratio increased by 29% and 32% when the KW PS was reduced from 5 mm to 2 mm and 5 mm to 1 mm respectively. It is possible that due to fractionation the solids reject from the sieve when the PS were reduced, influenced the detainment of some of the elemental components, thus, altering the elemental characteristics of the smaller PS.

According to the *p*-values from two sample t-tests conducted at α=0.05 (Table IV), reduction in KW PS from 5 mm significantly affected the elemental characteristics especially the carbon and nitrogen content. However, further reduction in PS from 2 mm to 1 mm did not significantly affect the elemental characteristics (except for hydrogen). The significant changes in elemental composition observed in the KW sample following PS reduction can be attributed to the fact that these elements are largely chemically bound within the solids.

Table IV. P-values of from two sample t-test analysis of elemental characteristics of KW sample at different PS

PS interaction	**N**	**C**	**H**	**C/N**
5 mm vs 1 mm	0.000	0.009	0.017	0.000
5 mm vs 2 mm	0.000	0.002	0.164	0.001
2 mm vs 1 mm	0.896	0.093	0.014	0.086

3.1.2 Volatile fatty acids profile

Considering that each experiment for the respective PS were set up differently with differing initial VFA concentration, the VFA degradation profile was normalised against the initial concentration on the day of set up (Day0) as shown in Figure 1. Hence, each experiment had a starting value of 1 and higher values in any experimental setup could imply either of two things; (i) the rate of VFA consumption was lower than the rate of VFA accumulation; such that, an increased rate of VFA consumption would bring this value closer to or lower than 1 and (ii) the amount of VFA produced during fermentation was relatively higher; such that, the higher values become more a function of initial VFA produced.

The latter implies that such reactors would yield more methane if all the VFA were eventually consumed. But this was hardly the case with higher food waste PS (especially 5 mm), which although had the highest VFA peaks, produced the least amount of methane (see Section 3.1.3). Therefore, the reduction in PS is believed to have influenced faster VFA consumption, according to the former assumption.

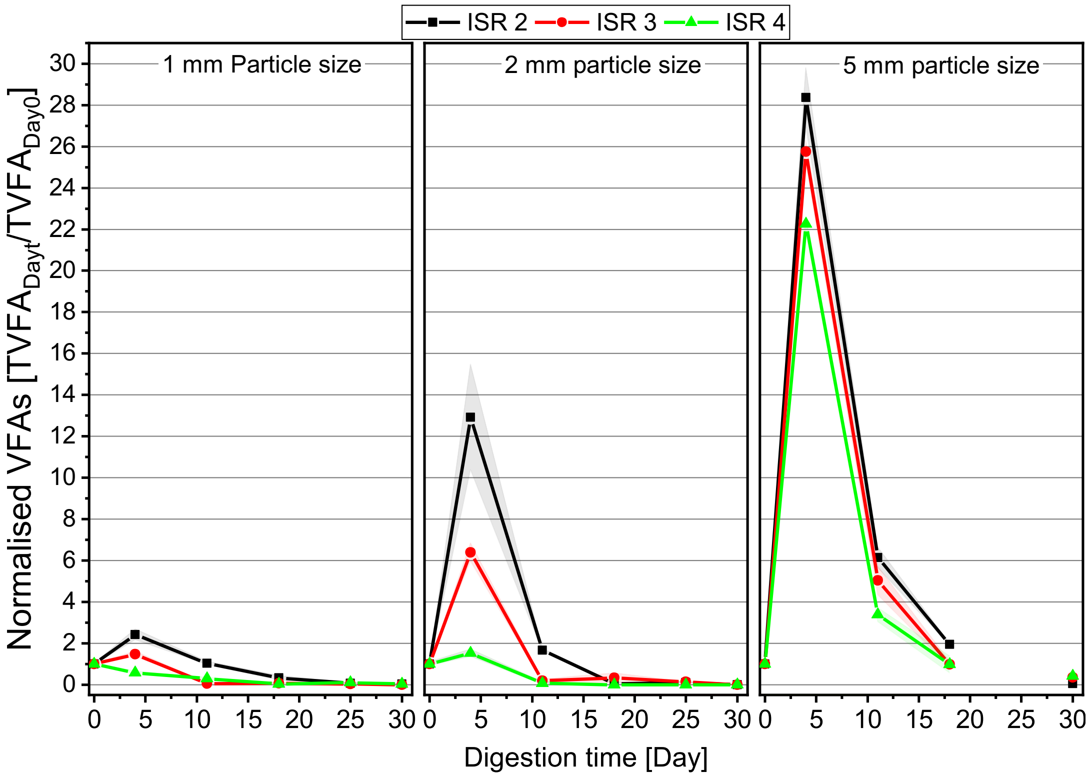

Figure 1. Total VFA degradation curves for PS and ISR optimisation experiments, normalised against the initial concentration at Day0. Disconnection between Day 30 and the rest of the data sets was due to missing data as a result of lab closure for that time period. Shaded area around lines represent standard deviation from mean.

In Figure 1, we observe that VFA accumulated up to as much as 30 times the starting concentration when 5 mm PS was employed. This reduced significantly with 2 mm PS treatment, which had VFA accumulation measuring up to 13 times its starting concentration. Further reduction to 1 mm PS resulted in VFA accumulating only less than 3 times its initial concentration. This is also supported by the lag in methane production within the early days of digestion at 5 mm PS for each corresponding ISR (discussed in Section 3.1.4). This means with 5 mm PS, methane production progressed at an ‘inhibited steady-state’; whereby, the process continued at a stable rate, but with low methane production (Angelidaki et al., 2016). It was not surprising to observe higher VFA accumulation at lower ISR for all three PS in the ISR order 2 > 3 > 4. Considering lower ISR meant relatively more food waste loading within the same PS experiments, the VFA levels increased at lower ISR during fermentation. The variation in ISR within each PS treatment was beneficial in identifying possible PS and ISR combinations that could help decrease the lag in methane production.

Acetic (A) and propionic (P) acids are the main precursors to methane production (Zhang et al., 2014). To minimise the VFA-induced inhibition, a P/A ratio of 1.4 have been set as a benchmark (Buyukkamaci and Filibeli, 2004; Marchaim and Krause, 1993). The P/A trends for all BMP assays are shown in Figure 2.

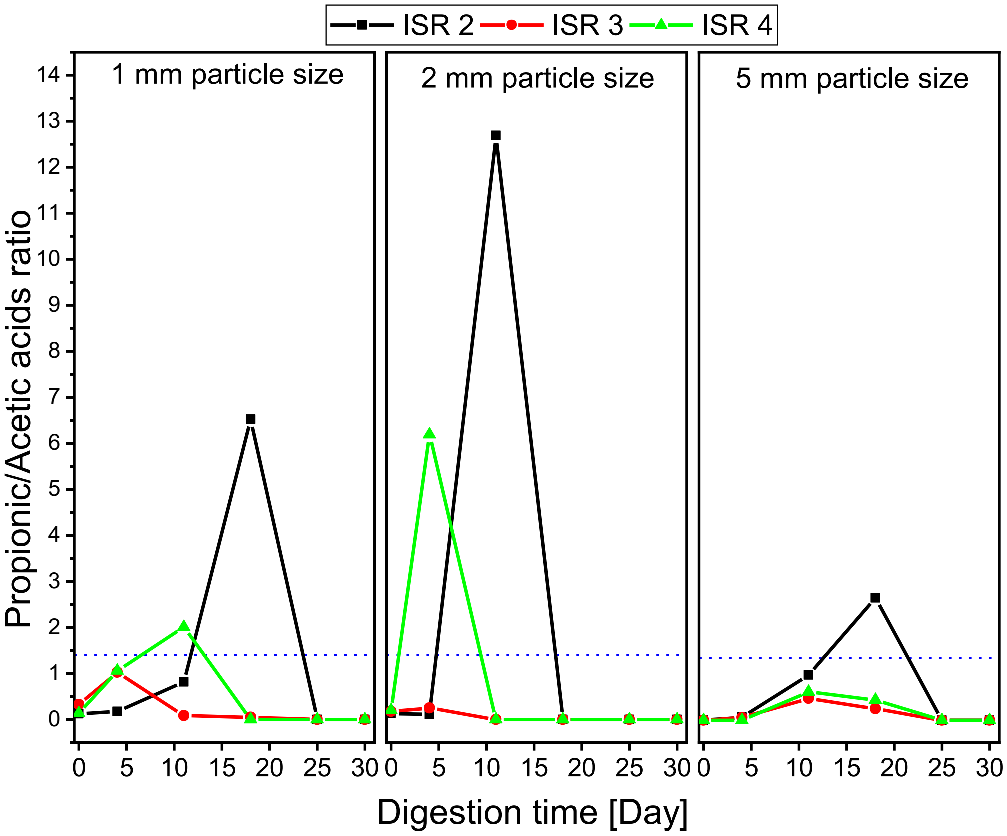

Figure 2. Propionic to acetic acid ratios for PS and ISR optimisation experiments using the grab sample; dotted lines indicate the acceptable limit of 1.4.

While the total VFAs at lower particle sizes of 1 mm and 2 mm were relatively lower than the levels measured at 5 mm PS (Figure 1), the corresponding P/A ratios at lower particle sizes were comparatively higher than the levels measured at 5 mm (Figure 2). This suggests that acetic acid degradation progressed at a faster rate than acetogenesis for lower PS of 2 mm and 1 mm, which is also supported by relatively lower lag times.

The P/A peaks observed at ISR 2 relative to ISR3 and ISR4 for all PS ranges could be due to the higher food waste loading at that ISR compared to the other ISR assayed. Interestingly, for 1 mm and 2 mm PS, the P/A levels at an ISR of 4 rose slightly above the threshold of 1.4. This was possibly due to a higher rate of acetic acid degradation following a higher availability of microorganism at that ISR.

Therefore, with PS reduction, the rate of acetic acid degradation was perceived to be increased, which is also supported by lower lag times recorded for smaller PS compared to a PS of 5 mm (Section 3.1.4) and at an ISR of 3, the P/A level was maintained below the threshold value at all PS.

3.1.3 Biomethane yield from Experiment 1

The biomethane yield from Experiment 1 ranged from 393 $\mathrm{NmLCH_4\ gVS^{-1}_{added}}$ to 543 $\mathrm{NmLCH_4\ gVS^{-1}_{added}}$ (Figure 3). The highest biomethane yield was obtained with a combination of 1 mm PS and 3:1 ISR, while the least yield was obtained with a combination of 5 mm PS and 4:1 ISR. The methane yield from this study is similar to values reported in literature in the range of 211 to 581 mL $\mathrm{CH_4\ gVS^{-1}_{added}}$, for food-based anaerobic digestion (Pham *et al*., 2015; Raposo *et al*., 2006; Zhang *et al*., 2013). From Figure 3, we observe that the high biomethane yields were obtained at 1 mm PS and decreased with increasing PS. This suggests that PS reduction does affect the BMP from food waste and is believed to be related to the improved VFA degradation rate. An overall biomethane increase of 38% was observed in this study with PS reduction. Similarly, Mshandete *et al*. (2006) reported 23% increase in methane yield from sisal fibre waste when it was reduced from 100 mm to 2 mm. Izumi *et al*. (2010), also stated that smaller mean PS of food waste increased overall methane yield by 28%, when the mean PS was reduced from 0.843 to 0.391 mm using a bead mill, because of enhanced solubilisation. In a study on the effect of PS and sodium ion concentration on anaerobic thermophilic food waste digestion, Kim *et al*. (2000) concluded that PS is one of the most important factors of food waste anaerobic digestion. Furthermore, they observed an inverse relationship between food waste and maximum substrate utilisation rate, with PS reduction from 2 mm to 1.02 mm. Although, these studies were conducted at largely varied PS ranges, they all attributed PS reduction with increase in biomethane yield due to enhanced substrate solubilisation.

Arguably, PS reduction would seemingly increase the energy demand in AD systems, however, at the time of conducting this study, there was no data on energy required for PS reduction to support whether the increased energy output achieved in this study can sufficiently cover the energy input. Nevertheless, a potential increase in methane yield such as the one obtained in this study, could increase the energy output to make up for the energy demand from size reduction. For instance, the gross calorific value of methane is 39.8 MJ $\mathrm{m^{-3}}$, as such, the energy value of the methane yield from 5 mm and 1 mm PS was 76,376 and 109,649 MJ $\mathrm{tonne^{-1}}$, equivalent to 21,216 and 30,458 kWh $\mathrm{tonne^{-1}}$ respectively (where 1 kWh = 3.6MJ). The efficiency for methane conversion to electricity was estimated to be 35% (Scarlat *et al*., 2018), hence, without further PS reduction (5 mm), an energy output of 7,426 kWh $\mathrm{tonne^{-1}}$ can be obtained. Meanwhile, with further PS reduction to 1 mm, the energy output increases to 10,660 kWh $\mathrm{tonne^{-1}}$, which is 43.5% higher than the energy output at 5 mm.

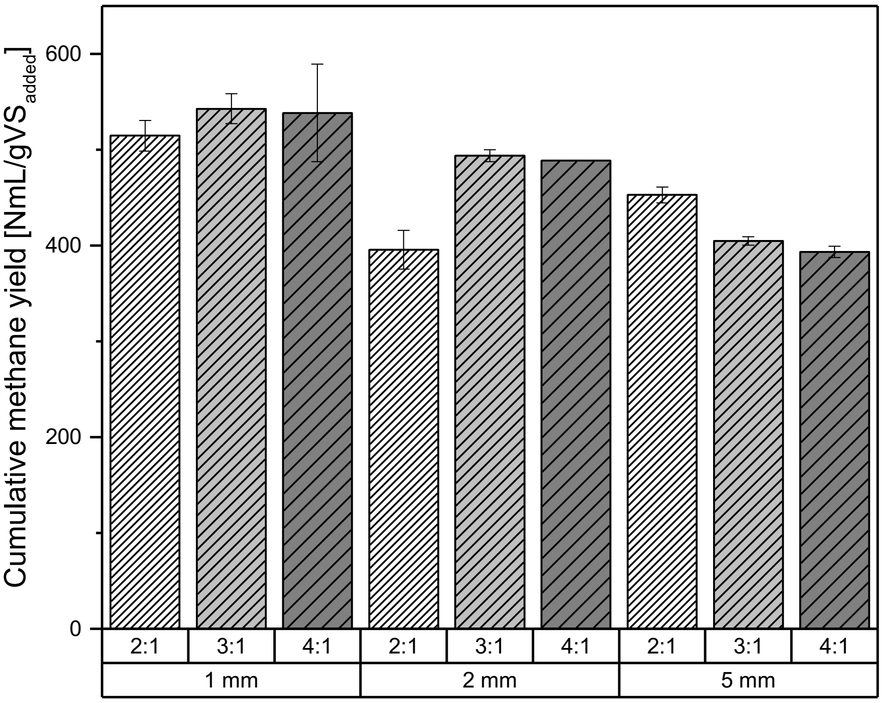

Figure 3. Overall methane yield from Experiment 1, with error bars indicating standard deviations.

Biomethane yield increased when the ISR was increased for smaller PS of 1 mm and 2 mm, while the opposite was observed at 5 mm PS. From the VFA profiles presented in Section 3.1.2 and the cumulative methane yield in Figure 3, it might be useful to accompany PS reduction with ISR increase for improved yield. This is because reducing the PS results in enhanced solubilisation; owing to an increased surface area. Consequently, the microorganisms (inoculum) should be increased to consume the high amount of solubilised materials. This factor is often neglected, which could be responsible for the contrasting findings by different studies on ISR and food-related waste BMPs. For instance, in a study with soybean curd residue - SCR (or okara), Zhou *et al*. (2011) reported an increase in methane yield with an increase in ISR, while Raposo *et al.* (2006) concluded there was no significant difference in the methane production coefficient from the BMP of maize at ISR 3, 2, 1.5 and 1 respectively.

3.1.4 Kinetic assessment

The MGompertz model was used in fitting the experimental data, being widely adopted for fitting cummulative methane production (Kong *et al*., 2016; Pellera and Gidarakos, 2016; Lü *et al*., 2015; Moset *et al*., 2015; Mottet *et al*., 2014; Pan *et al*., 2013; Wall *et al*., 2013; Boulanger *et al*., 2012; Xie *et al*., 2011; Shin *et al*., 2004; Hong and Wrolstad, 1990). In agreement with the VFAs profile (Section 3.1.1), reduction in lag time was observed when PS was reduced from 5 mm to 2 mm and 1 mm (Table V), as a result of an increase in the degradation rate. Although, shorter lag times were observed with PS 2 mm, it did not necessarily culminate in the highest methane yield. Thus, it can be inferred, that combining a low PS (such as 1 mm and 2 mm) with a low ISR of 2:1 might not be suitable due to an increase in lag time. A similar effect was observed with the combination of high PS of 5 mm and a high ISR of 4:1. Overall, the lag time reduced from 7 days with 5 mm PS to as low as 0.1 day with PS reduction. Hence, the choice of PS and ISR could greatly impact the kinetic parameters for food waste anaerobic digestion.

Table V. Particle Size (PS) and Inoculum-to-Substrate ratio (ISR) influence on process kinetics and biodegradability.

PS	**ISR**	***k*-value (Day^{-1})**	**R^2**	**Lag phase (Day)**	**Theoretical methane potential (NmLCH$_4$ gVS$^{-1}_{FW}$)**	**Experimental yield (NmLCH$_4$ gVS$^{-1}_{FW}$)**	**Percentage biodegradability (%)**
1 mm	2:1	0.27	0.99	3.5	515.65	514.63	99.8
	3:1	0.43	0.99	0.2	515.65	542.79	105.3
	4:1	0.40	0.98	0.4	515.65	538.33	104.4
2 mm	2:1	0.33	0.99	0.9	483.91	395.73	81.8
	3:1	0.53	0.99	0.1	483.91	493.84	102.1
	4:1	0.74	0.99	0.1	483.91	488.47	100.9
5 mm	2:1	0.25	0.98	5.8	547.90	452.89	82.7
	3:1	0.39	0.99	6.3	547.90	404.72	73.9
	4:1	0.46	0.99[a]	7.0	547.90	393.42	71.8

The overall percentage biodegradability was highest at 1 mm PS and ratio 3:1. Based on the results shown in Table V, it is possible to infer that PS reduction improves the anaerobic biodegradability of food waste and hence, the ability to better assess methane production under BMP test conditions. Nielfa *et al.* (2015) also reported similar high percentage degradability (≥100%) for organic fraction of municipal solid waste.

3.1.5 Statistical analysis

A response surface regression was conducted for the cumulative methane yield versus the ISR using obtained yields from the 3^2 factorial DOE (n=18) to establish the Equations 2 to 4 (where P = PS). These equations were then used to predict the cumulative methane yields at PS 3 mm and 4 mm shown in **Error! Reference source not found.**.

$$Cumulative\ methane\ yield\ at\ ISR\ 2:1 = P^2 - 6.74P + 39.08 \qquad \text{Eq. 2}$$

$$Cumulative\ methane\ yield\ at\ ISR\ 3:1 = P^2 - 8.52P + 45.60 \qquad \text{Eq. 3}$$

$$Cumulative\ methane\ yield\ at\ ISR\ 4:1 = P^2 - 8.64P + 45.44 \qquad \text{Eq. 4}$$

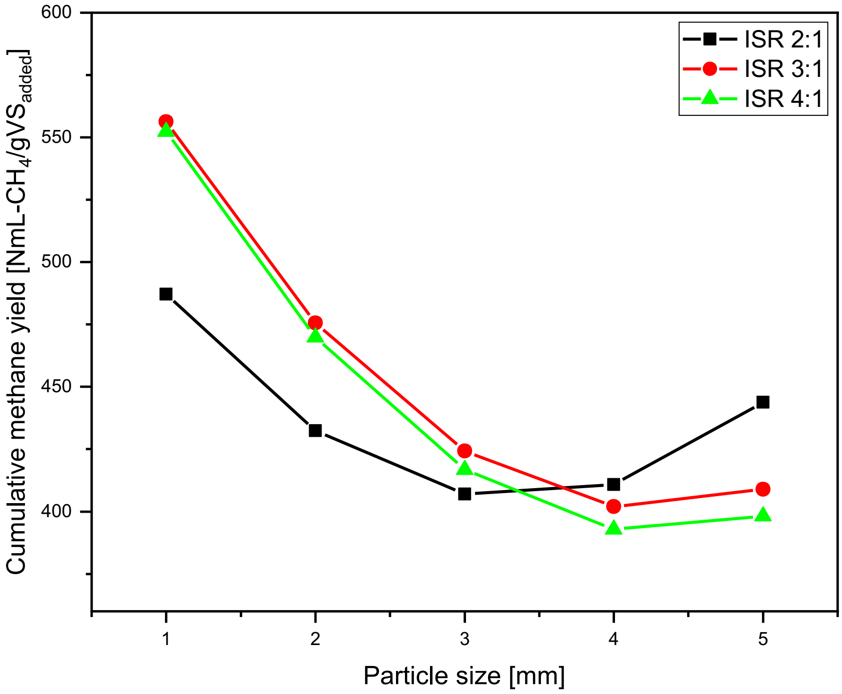

Figure 4. Interaction plot for cumulative methane yield versus food waste PS at different ISR

Figure 4 further demonstrates that increase in methane yield is inversely proportional to increase in PS at all tested ISR. The ISR of 3:1 enriched higher biomethane yield (especially at lower PS) than 2:1 and 4:1; the reason being a relatively balanced fraction of substrate to acting microbial load, which enabled non-inhibitory VFA production and consumption trend. It is established here that PS pre-treatment was the more influencing factor on the methane yield obtained.

3.2 Experiment 2: influence of trace elements concentration towards methane production

3.2.1. Food Waste and Inoculum contribution towards trace element content

According to Reilly (2002), the concentration and presence/absence of trace elements in food waste is a consequence of various factors, including environmental aspects, such as nutrient availability in soil. Therefore, for a better means of comparison, the trace elements present in CFW were juxtaposed to food waste samples from across the UK. Nevertheless, the values were significantly lower, and could be a result of the metal analysis methodology and/or sample composition, amongst other factors.

*Table VI. Trace elements on CFW, inoculum, nutrient media and recommended values for anaerobic biomass**

Element/Reference	Co mg/KgTS	Fe mg/KgTS	Ni mg/KgTS	Mn mg/KgTS	Mo mg/KgTS	Se mg/KgTS	W mg/KgTS
Food Waste (Composite Sample)							
Ludlow, UK 2015(a)	0.1	89	n.a.	92	0.37	0.17	n.a.
Ludlow, UK 1998(b)	>0.25	229	n.a.	85 (14)	0.46 (0.05)	>0.30	n.a.
Luton, UK(b)	0.07 (0.01)	148 (1)	n.a.	97.7 (1.6)	1.1 (0.2)	1.2 (0.6)	n.a.
Hackney, UK(b)	0.35 (0.19)	175 (58)	n.a.	94.5 (4.1)	1.2 (0.2)	0.4 (0.3)	n.a.
Present Study	0.030 (0.005)	4.2 (0.6)	0.20 (0.03)	0.60 (0.08)	0.030 (0.004)	0.4 (0.1)	0.005 (0.001)
Inoculum							
Facchin *et al*. (2013)	2.9	n.a	24.2	n.a	4	<1	2.7
Banks *et al*. (2012)	0.083	n.a	2.9	n.a	0.29	0.050	<0.035
Present Study	0.003	n.a	0.01	n.a	0	0.03	0.002
Recommended – Anaerobic Biomass							
Facchin *et al*. (2013)	9	-	11	-	7	1.5	<0.1

(a) Yirong *et al*. (2015); (b) Hansen *et al*. (1998).
*Figures are reported s mean values with standard deviation is in brackets, when available
n.a. - not analysed.

The inoculum used for this experiment showed values for most metals below range of those reported elsewhere in the literature for seeds treating food waste (Table VI). The trace element content from the inoculum is a relevant information, since it can sometimes counter-balance the lack of nutrients presents on food waste, thus stabilizing the anaerobic digestion process (Qiang *et al*., 2012). Based on the recommended concentrations of the trace metals for anaerobic biomass by Facchin *et al*. (2013), it is clearly seen from Table VI that the CFW sample would not provide enough nutrient content on its own for the biomass, even with the inoculum contribution, corroborating that the sample could benefit from nutrient media supplementation.

Therefore, the amount of trace elements to be added was determined by the combination of different metal mixtures (Adewale, 2014, Angelidaki *et al*., 2009, Owen *et al.,* 1979 and Kim *et al*., 2003) as an attempt to supply the biomass with all the necessary nutrients for the stable anaerobic digestion process. Differently from previous studies in the literature, there was no individual metal concentration value calculation to meet the specific requirements of the studied food waste sample.

3.2.2 Process stability and methane yield in the batch trial under media supplementation

The nutrient media supplemented reactor exhibited a more stable anaerobic digestion of food waste when compared to the control (no media supplementation) (Figure 5a). The absence of sharp pH drops because of no VFAs accumulation during fermentation (expected to be intensified on the first week of digestion), demonstrates the possible benefit of nutrient supplementation. As opposed to the control where an uncoupling between production and consumption of VFAs occurred, resulting in its accumulation and simultaneous pH drop between day 4 and 7. The control behaviour was already anticipated, as the single stage anaerobic digestion performance of food waste is usually reported as unsuccessful, mainly due to the rapid consumption of the labile fraction of the waste, which ultimately leads to the described scenario (Ma *et al.*, 2011).

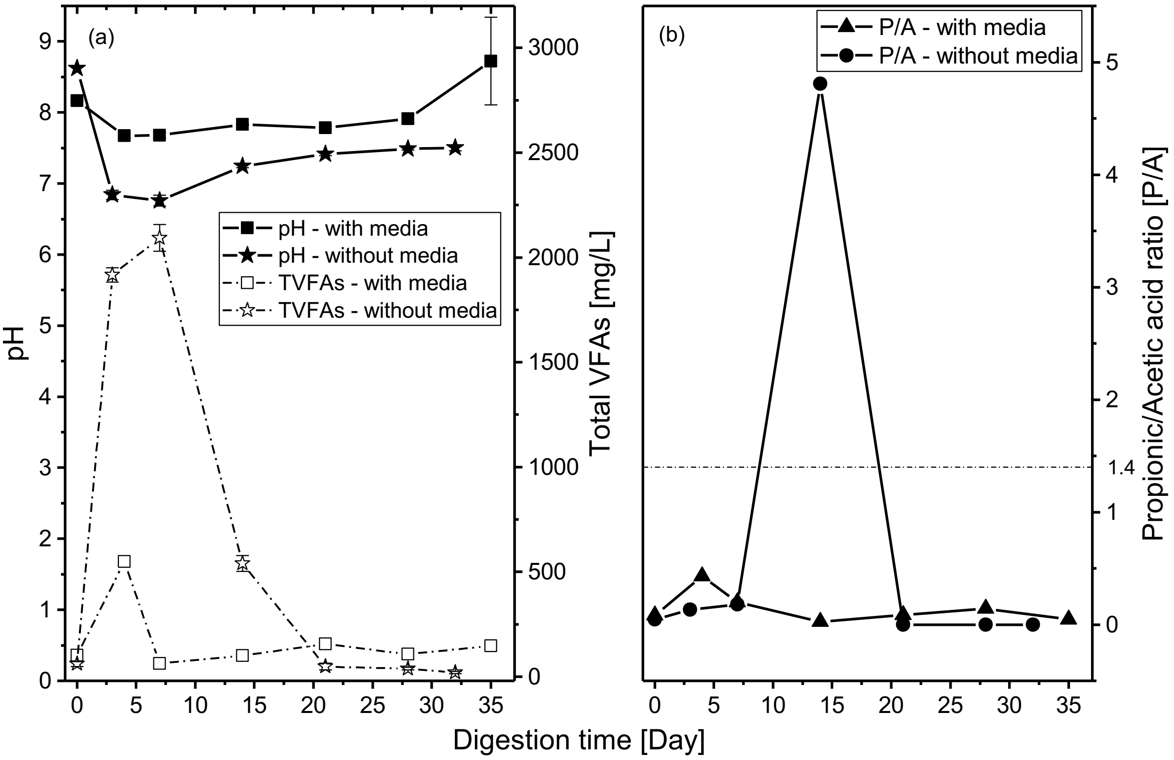

Figure 5. a) Total VFAs concentration and pH for the nutrient media supplemented reactor and control (no nutrient media supplementation); b) Propionic to Acetic Ratio for the nutrient media supplemented reactor and control.

Zhang *et al*. (2012) treated food waste on a single-stage mesophilic anaerobic digestion and demonstrated that when supplemented with Co, Fe, Mo and Ni, the digestion became more stable in terms of pH values and lower VFAs levels when compared to the control, suggesting that these nutrients have an important role for improving methanogens and the overall process performance. Similarly, in this study, the total VFAs levels were also higher for the control than for the supplemented reactor between day 4 and 7, where a concentration of 2,101.4 mg L^{-1} was observed as opposed to only 548.7 mg L^{-1} for the same period in the nutrient treated reactor. This substantiates the rapid consumption of the readily degradable fraction of food waste faster in a nutrient balanced digestion, as well as the maintenance of a lower concentration levels of VFAs by the presence of certain metals.

As previously mentioned, P/A ratio can be used as a tool for detecting digestion imbalance, with values above 1.4 suggesting digester failure (Hill *et al*., 1987). On the fourteenth day of experiment the control showed a P/A of 4.6 (Figure 5b). Conversely, the reactor supplied with nutrient media did not show any P/A values above 1.4 throughout the digestion period (Figure 5b). According to Qiang *et al*. (2013), when the digestion of food waste is nutrient-sufficient, the propionic acid degradation rate is constant and therefore, there is no VFAs/propionic acid accumulation. On the contrary, under insufficient amounts Ni, Co and Fe, the anaerobic digestion becomes unstable, thus more susceptible to failures. Additionally, Banks *et al*. (2012) concluded that

Se and Mo and W are essential when performing batch trials of mesophilic anaerobic digestion on food waste, improving the acetic and propionic acid degradation respectively.

It is clearly seen from the results that although the composite food waste sample and the inoculum used in this study did not provide enough concentration of nutrients for the anaerobic biomass, the trace elements supplementation in a form of a pre-determined media, containing amongst other elements: Co, Mo, Fe and Ni counterbalanced the lack of nutrients. This offered protection against VFAs accumulation/ propionic acid build-up, hence, avoiding a likely esteemed digestion failure.

The cumulative methane yields for the reactors with and without the influence of nutrient media supplementation is depicted in Figure 6. Notably, the control exhibited higher methane yield (544.6 NmL gVS^{-1}_{added}) compared with the supplemented reactor (490.5 NmL gVS^{-1}_{added}). However, methane production rate differed significantly between them, with the nutrient media supplemented reactor presenting a much faster rate than the reactor without media on the first days of anaerobic process. This behaviour was already expected, as the VFAs accumulation between the 4 – 7th days of digestion negatively influenced methane production for the same period in the reactor without media. For this reason, methane production was hindered, only significantly increasing from the 8th day of digestion, as opposed to the media supplemented reactor, in which the first week was the most relevant period for methane generation. The improved process performance in this case is also confirmed by the technical digestion time (T80), which corresponds to the period (in days) taken by the digestion process to achieve 80% of the cumulative yield (Xie *et al.*, 2011). The nutrient supplemented reactor, reached the T80 at the 7th day of digestion, as opposed to the reactor without media, which only reached at the 18th day, representing a 2.57 times faster rate, when the process is under nutrient-sufficient conditions.

The observed delay of methane production for the control at the first week of digestion was also reflected on the lag phase, which was 11.58 times longer than for the nutrient enriched reactor; once more validating the better performance of the anaerobic digestion of food waste on the first week when nutrient media was added (Table VII). Additionally, the process kinetics for the control was also negatively affected, exhibiting a *k*-value of 0.215, equivalent 2.10 times lower than the nutrient enriched reactor.

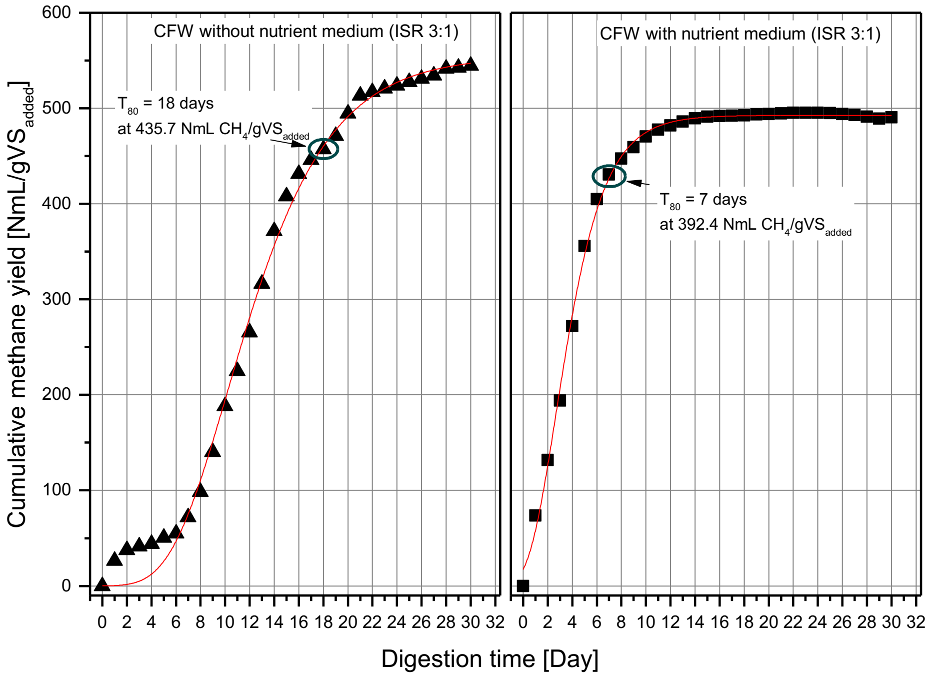

Figure 6. Cumulative Methane yield (dotted points) with Modified Gompertz fitting (red line) for CFW with and without media supplementation, showing the influence of nutrient media on attaining T80.

Table VII. *Kinetics for experiment 2.*

Sample	ISR	*k*-value (Day^{-1})	R^2	Lag phase (Day)	Theoretical potential (NmLCH$_4$ gVS^{-1}$_{FW}$)	Experimental yield (NmLCH$_4$ gVS^{-1}$_{FW}$)	Percentage biodegradability (%)
Nutrient media supplemented	3:1	0.45	0.998	0.5	588.78	490.48	83.3
Control	3:1	0.22	0.994	5.6	588.78	544.62	92.5

Biodegradability rate of the different conditions were analysed according to (Speece, 2008). As it can be seen from Table VII, biodegradability was not related to the process stability, but to its performance (methane yield). Therefore, the reactor without media showed the highest percentage biodegradability than the nutrient enriched reactor, meaning that the experimental values obtained by the BMP test were closer to the theoretical methane values obtained by Buswell equation (Buswell, 1952).

4. Conclusions

The results presented in this paper suggests that PS reduction improved the anaerobic degradability of food waste, which consequently improved the assessment of methane production under BMP test conditions. Although, excessive food waste PS reduction increases the tendency for VFAs build-up, this was overcome by a proper selection of ISR, thus, stabilising the digestion process and avoiding this common finding when anaerobically digesting food waste as a sole substrate, in a single-stage process.

For smaller PS of 1 mm and 2 mm, a combination with an ISR of 3:1 and 4:1 helped to stabilise the systems, while with larger PS of 5 mm, an ISR of 2:1 was most suitable. Consequently, lower lag times were observed at ISR of 3:1 and 4:1 for 1 mm and 2 mm PS treatments and at ISR of 2:1 for 5 mm PS respectively. In general, for PS ≤3 mm the highest methane yield was obtainable at ISR of 3:1, while for PS ≥3 mm, the highest methane yield was obtainable at ISR 2:1. As a result of improved degradability and a balanced PS and ISR combination, an overall methane increase of 38% was obtained with a PS reduction from 5 mm to 1 mm, which corresponds to a potential rise in the energy output from 7,426 kWh tonne^{-1} to 10,660 kWh tonne^{-1}.

Differently from the combined PS reduction and ISR effects, which heralded a positive effect on the final methane yield of food waste, nutrient media supplementation did not enhance the ultimate methane yield. On the other hand, it was found that its application helped to stabilise food waste digestion process by avoiding: a) VFAs accumulation and high P/A ratio and b) reducing the lag time (8.9% less time needed), thus strongly suggesting that nutrient media supplementation could significantly reduce the hydraulic retention time (HRT) of food waste anaerobic digestion, thus increasing the throughput and biomethane recovery.

Further investigation needs to be done on the bioavailability of essential nutrients such as Ni, Co, Mo, Se, W, Fe and Mn during the digestion process of food waste, hence, enabling a better understanding of these nutrients utilisation in batch systems, offering a possibility for further adjustments and improvement of the media here tested.

As documented by this study, there is not a clear winner strategy for methane yield enhancement from food waste as a sole substrate in AD. All the applied methods (PS, ISR and nutrient media), have benefits, and costs related to energy input that need to be estimated for large scale operational systems. However, the authors believe that the findings here discussed could benefit the AD industry by emphasising the importance of better testing conditions and combining already existing methods to try to maximise this sector efficiency.

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