Accepted Manuscript


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PII: S2589-014X(18)30114-2
DOI: https://doi.org/10.1016/j.biteb.2018.11.004
Reference: BITEB 113

To appear in: *Bioresource Technology Reports*

Received date: 12 September 2018
Revised date: 10 November 2018
Accepted date: 11 November 2018

Bioresource Technology Reports, Volume 5, February 2019, pp. 243-250
DOI:10.1016/j.biteb.2018.11.004

# Evaluation of anaerobic digestibility of energy crops and agricultural by-products

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## Abstract

Abundant wastes from the food and drink supply chain are valuable and infrequently used as anaerobic digestion (AD) substrates. This study quantifies their biomethane potential to contribute to solid waste reduction and energy production. 29 organic materials were evaluated: energy crops (6), pre-treated agricultural by-products (5), livestock slurries (3), agro-industrial wastes (7), fruit and vegetable wastes (4) and co-digestion mixtures of chicken litter (CL) and fruit wastes (4). Results showed highest biogas yields for rendered fat washings (1379 ± 125 mL/g $VS_{feedstock}$), fish waste (898 ± 107 mL/g $VS_{feedstock}$) and potato waste (768 ± 27 mL/g $VS_{feedstock}$). Synergistic benefits of co-digestion were evidenced. CL (20%) with avocado pulp (80%) led to 84% higher biogas than expected from contribution of single substrates.

## Keywords

Agricultural by-products; energy crops; co-digestion; livestock manure; anaerobic digestion

## 1. Introduction

In 2015 the world energy supply was 13,647 million tonnes of oil equivalent and it is predicted to continue increasing due to population growth and advancement of developing countries (IEA, 2017a). However, concern over reserves and over their environmental impact is driving developments on renewable sources of energy. Renewable energy currently accounts for 13.4% of the total world production while petroleum and other liquids, natural gas and coal comprise the majority (IEA, 2017b).

The United Kingdom (UK) used 17,296 thousand tonnes of oil equivalent of renewable fuels in 2016 which accounted for 12% of the country's total energy consumption (UK Department for Business, 2017). The UK continues to progress in terms of using renewable energy sources and have already exceeded their Renewable Energy Directive target (UK Department for Business, 2017). Of the alternative options, anaerobic digestion (AD) plays a key role: it recovers valuable products, such as nutrients, reduces carbon dioxide ($CO_2$) emissions, and yields renewable energy.

In the past, AD plants typically used sewage sludge to yield biogas but in more recent years the AD industry has enhanced gas production by employing energy crops and food wastes (FW) as a feedstock . Currently, there are over 300 digesters in the UK operating with feedstocks other than sewage sludge (USDA, 2018) and biogas generated in ADs treating substrates other than sewage sludge is predominant in several European countries (EurObserv'er, 2017).

Despite the increase in biogas production from alternative substrates, there is a drive to identify alternative feedstocks to enhance renewable energy production, either by mono digestion of single waste streams or by co-digesting with feedstocks such as FW and sewage sludge (Chiu and Lo, 2016). A promising alternative source is the agricultural sector, which is responsible for one of the largest waste streams; generating natural wastes such as manure and arable by-products that could be used for renewable energy generation by AD. To illustrate, nearly 64 million tonnes of fish waste is generated annually worldwide, most of which is underutilised as low-value animal feed (Nges et al., 2012). Avocado processing generates ca. 18,144 tonnes of avocado waste annually for Mexico alone (Clarke et al., 2008; Siddiq et al., 2012). Both in the UK and the United States (US) chickens are the most farmed livestock followed by beef cattle and dairy cows (USDA, 2018). All of which constitute examples of waste sources that, if treated by AD, could significantly reduce disposal costs and the amount of uncontrolled methane ($CH_4$) being released to the atmosphere, with concomitant increases in renewable energy production. There are previous studies available assessing the biodegradability of energy crops and agricultural by-products (Table 1). However, a single study that allows comparison of methane yields and formation kinetics for a wide range of substrates, performed with the same laboratory procedure, is still lacking.

This study investigates the benefits of anaerobically digesting a wide variety of energy crops and agricultural by-products, as single substrates or as co-digestion. 29 different organic materials were selected and its biogas formation potential (yield and kinetics) evaluated: energy crops (6), pre-treated agricultural by-products (5), livestock slurries (3), agro-industrial wastes (7), fruit and vegetable wastes (4) and co-digestion mixtures of chicken litter (CL) and fruit wastes (4).

## 2. Materials and methods

### 2.1. Feedstock and inoculum

The inoculum used in all tests was the effluent from a FW AD plant located in Southwest England and treating 80,000 tonnes of FW per year. All substrates were collected from May to July 2017. All energy crops obtained were harvested in their milky stage, manually cut to approximately 2 cm and stored at 4°C until the moment of use. Grass was collected in its young leaf stage. Straw was collected before silage. Untreated wheat straw was manually cut to approximately 2 cm while pre-treated wheat straw was steam treated at 4, 6, 8 and 10 bar with temperatures ranging from 140°C (4 bar) to 180°C (10 bar) using a continuous pressurised refiner (Andritz Sprout-Bauer, Denmark) and then kept frozen until used. CL contained small amounts of bedding (consisting of sand, wood chips and straw bales) and cracked egg shells. The young chick litter, cow manure, onion waste and potato waste were all obtained from the same farm (Table 2). The young chick litter was collected from the tray beneath the cages and contained a small amount of feed. Cow manure was stored outside on the farm and contained wood chips and residual onion from their diet and a small amount of straw from the bedding. Onion waste consisted of peels, onion juice and damaged onions which are used to feed cattle on the farm along with potato waste. Potato waste, originating from potato processing, was stored outdoors while on site and was contaminated with small amounts of onion waste. Onion waste and potato waste were not further processed before being anaerobically digested. Cheese whey originated from blue cheese production. The spent grain was collected from open containers located outside the brewery and waste yeast was collected from a refrigerated, air tight container. Sugar beet tops were processed with a meat mincer to a particle size below 3 mm. All substrates were stored at 4°C since collection until use, with the exception of wheat straw that was frozen. The banana peels (BP) used in the batch tests were fully ripe and some were flecked with brown. BP were manually cut into approximately 1.5 $cm^2$. Fresh potatoes were peeled manually and the peels used for anaerobic digestion without further processing. Avocado seeds were tested for single substrate digestion experiments after being processed in a meat mincer to a particle size bellow 3 mm.

Waste over-ripe bananas were manually separated from the flesh. BPs, whole bananas (WB) and avocados pulp were processed (< 3mm particle size) through a meat mincer to homogenize the substrates before preparing the co-digestion blends on wet weight basis of 50:50 BP with CL (50:50-BP:CL), 50:50 WB with CL (50:50-WB:CL), 80:20 avocado pulp with CL (80:20-A:CL) and 50:50 avocado pulp with CL (50:50-A:CL) which were then stored at 4°C until further use.

### 2.2. Batch digestion experiments

Biomethane potential tests were conducted in 1 litre High-density polyethylene reactor bottles (Anaero Technology, Cambridge, UK) with a 700 mL working volume. The reactors were submerged in a water bath and maintained at 38°C in order to operate at mesophilic conditions. An inoculum to substrate VS ratio of 4:1 was used for all tests except soya (6:1), rendered fat washings (6:1) and CL (8:1). Inoculum was diluted for the rendered fat washings and cheese whey experiments (Table 2). BMP equipment (Anaero Technology, Cambridge, UK) normalised gas flow to standard temperature and pressure (STP) conditions (273°K and 1013.25 kPa). All reactors were continuously stirred at 45 rpm by a paddle mixer. A triplicate of inoculum without any feedstock was used in each set of tests as a control to determine the baseline gas production per gram of inoculum VS. All feedstocks were tested in triplicate except for: rye (whole-crop), soya (whole-crop), and untreated and pre-treated wheat straw which were performed in duplicate due to insufficient sample. Sampling on site was performed once for each substrate, with sub-samples of the collected feedstock used for test replication.

## 2.3. Analytical methods

All substrates were analysed for total solids (TS) and volatile solids (VS) according to the American Public Health Association (2005). A 1-L Tedlar gas bag was connected to the discharge point of the gas flow meter. Biogas produced in the batch experiments was collected for the first 15 days and composition ($CH_4$) was analysed once using a Geotech BIOGAS 5000 gas monitor.

Biogas yield expressed in mL/g VS added was calculated as the cumulative volume of biogas produced per g of VS added to the reactor then subtracting the average biogas yield obtained from the triplicate control reactors based on inoculum VS. The termination criterion for ultimate biogas yield was when daily biogas production was less than 5%. The longest time it took for a substrate to reach less than 5% daily biogas production was 20 days. All substrates yields are reported to 20 days.

### 2.3.1. Kinetic study

Lag phase (λ) was estimated using the modified Gompertz equation (Ghatak et al., 2014) fitted to experimental values and applying the Microsoft Excel Solver tool by performing a non-linear least-square regression analysis (3-1).

$$P = P_{max} \times exp\left\{-exp\left[\frac{R_{max} \times e}{P_{max}}(\lambda - t) + 1\right]\right\}$$ (Eq. 1)

Where, P is the cumulative biogas yield for a given time (mL/g $VS_{feedstock}$); $P_{max}$ is the biogas production potential (mL/g $VS_{feedstock}$); $R_{max}$ is the ultimate biogas production rate (mL/g $VS_{feedstock}$/d); λ is the lag phase (d); t is time (d) and e is a mathematical constant, exp (1), (e=2.178282). The Gompertz equation was applied as it is commonly reported to provide the highest correlation value for simulating cumulative biogas production in batch digestion as compared to the first order kinetic model.

Analysis of variance (ANOVA) was used to determine statistical significance with a threshold p-value of 0.05.

## 3. Results

### 3.1. Biomethane potential tests

#### 3.1.1. Characterisation of inoculum and substrates

The TS and VS of all substrates and inoculum tested in this study are shown in Table 2. Due to the wide range of substrates tested, the VS content of all substrates ranged from 0.2 to 69%, with rendered fat washings being the lowest and pre-treated straw at 6 bar the highest. The inoculum used in all tests had an average TS and VS content of 4.75±0.9% and 3.09±1.0%, respectively.

#### 3.1.2. Digestion performance: Biogas potential and kinetics

Biogas yield, $CH_4$ yield and $CH_4$ content of all substrates are shown in Table 3. Lag phase for each experiment calculated with Gompertz equation are also reported.

**a. Energy crops**

Digestion of grass obtained the highest biogas yield (622±127 mL/g $VS_{feedstock}$) within the energy crops tested, achieving its ultimate biogas yield in around 8 days (Figure 1). For ryegrass, triticale, rye, wheat and soya, the cumulative biogas yields were 481±34, 510±18, 432±19, 404±38 and 594±31 mL/g $VS_{feedstock}$, respectively. However, the $CH_4$ yield of soya was the greatest compared to all other energy crops tested (376±19 mL/g $VS_{feedstock}$). The $CH_4$ yields of the remaining energy crops varied between 283 to 359 mL/g $VS_{feedstock}$ (Table 3). It was observed that grass was the only energy crop to have a calculated lag phase of 0 days while triticale had the longest compared to all substrates tested, 6.8 days (Table 3).

**b. Pre-treated straw**

All pre-treated straw samples showed a similar biogas production rate on days 1 to 3 (Figure 1). However, the untreated straw had a longer lag period (4.9 days) in comparison to all pre-treated straw (4 bar: 3.1, 6 bar: 2.0, 8 bar: 3.0 and 10 bar: 2.9 days) (Table 3). The biogas yields for untreated straw, pre-treated straw at 4, 6, 8 and 10 bar were 326±22, 441±42, 313±17, 426±7 and 356±11 mL/g $VS_{feedstock}$, respectively. Before day 13 all substrates had produced approximately 80% of their ultimate cumulative biogas yield. It was observed that straw pre-treated at 4 bar had the longest lag phase (3.1 days) compared to other pre-treated samples but resulted in the highest $CH_4$ yield (271±26 mL/g $VS_{feedstock}$). In comparison, straw pre-treated at 6 bar had the shortest lag phase (2.0 days) but generated the lowest $CH_4$ yield (198±11 mL/g $VS_{feedstock}$). The $CH_4$ content of all straw digested ranged from 60.4 to 63.2% (Table 3).

**c. Livestock slurries**

The biogas yields of CL, chick litter and cow manure were 307±24, 371±23 and 123±32 mL/g $VS_{feedstock}$, respectively. By day 9, chick litter and CL had produced more than twice the amount of biogas that cow manure produced during the 20-day test duration (Figure 1). $CH_4$ content for all livestock substrates was similar (59.9, 61.8 and 61.6% for CL, chick litter and cow manure, respectively) (Table 3).

### d. Agro-industrial waste

Rendered fat washings experienced a lag phase of 5.3 days but had a significantly higher cumulative biogas yield (1,379±125 mL/g $VS_{feedstock}$) in comparison to all other substrates tested. Its yield was *ca.* 54% more than the next highest yielding substrate, fish waste (898±107 mL/g $VS_{feedstock}$). Similarly, cheese whey generated a biogas yield of 818±10 mL/g $VS_{feedstock}$. Comparing the two brewery wastes, spent grain generated a higher biogas yield (644±42 mL/g $VS_{feedstock}$) than waste yeast (412±33 mL/g $VS_{feedstock}$) which reached its full biogas potential by day 6. Sugar beet tops produced 95% of its total biogas potential by day 5, earlier than all other agro-industrial substrates tested (Figure 1). A lag phase was observed for potato waste until day 6 and a biogas yield of 768±27 mL/g $VS_{feedstock}$ was recorded. $CH_4$ content of fish waste, potato waste, sugar beet tops, whey, spent grain and waste yeast was 60.7, 51.7, 65.5, 43.9, 60.5 and 53.3%, respectively. The $CH_4$ content of whey was the lowest out of all substrates tested although its $CH_4$ yield was 359±4 mL/g $VS_{feedstock}$.

### e. Fruit and vegetable waste

The avocado seed reached its ultimate biogas potential much earlier than potato peels and banana peels, producing 90% of its total biogas potential by day 7 (Figure 1). Biogas yields (mL/g $VS_{feedstock}$) were 568±17, 633±7, 474±23 and 576±15 for BPs, potato peels, avocado seed and onion waste, respectively. Potato peels exhibited a shorter lag phase in comparison to potato waste (3.7 and 6.2 days, respectively) but generated a lower $CH_4$ yield (331±3 mL/g $VS_{feedstock}$). Onion waste performed the highest in terms of $CH_4$ content, yielding 62.5% and after a lag phase of 6.5 days (Table 3).

### f. Co-digestion

Biogas yields of 80:20-A:CL, 50:50-A:CL, 50:50-BP:CL and 50:50-WB:CL were 811±19, 602±94, 425±16 and 625±3 mL/g $VS_{feedstock}$, respectively (Table 3). 80:20-A:CL and 50:50-WB:CL produced the highest $CH_4$ yields of all co-digested substrates (517±13 and 422±46 mL/g $VS_{feedstock}$, respectively). 50:50-WB:CL and 50:50-BP:CL exhibited similar initial (2.2 and 1.9 days, respectively) before producing 95% of their ultimate biogas potential before day 5. Both avocado mixtures showed a steady production of cumulative biogas during the 20-day test. $CH_4$ content of all co-digested mixtures ranged from 63.8 to 67.5%.

## 4. Discussion

### a. Energy crops

Energy crops are recognised for providing substantial $CH_4$ yields in contrast to sewage sludge or livestock slurries. Many AD plants in the UK operate with one energy crop as a feedstock; commonly maize, whole-crop cereals and grass silage. In general, energy crops have a recalcitrant structure, with high contents of lignin, cellulose and hemicellulose (Croce et al., 2016; Theuretzbacher et al., 2015). Lignin content covers the hemicellulose and cellulose layers preventing contact from enzymes, consequently preventing hydrolysis (Ferreira et al., 2014) and resulting in slow biogas production. This gradual digestion is observed in the batch assays for triticale, wheat and rye which had the longest calculated lag phases (6.8, 5.6 and 3.5 days, respectively). It is probable they contain greater amounts of lignocellulose as compared to ryegrass, grass and soya which all exhibited faster digestion times (2.6, 0.0 and 1.8 days calculated lag phase, respectively). In this study, soya was harvested early before beginning bloom which could have decreased the proportion of lignocellulosic structures allowing for it to be degraded more easily. Cellulose fibres are difficult for bacteria to degrade when they are covered by lignin which prevents access to the cellulases. Biogas production of lignocellulosic biomass is influenced in several ways such as, biochemical composition, harvesting season and particle size (Amon et al., 2007). No studies have been reported on the digestion of whole-crop soya. However, all other substrates tested were in accordance to yields reported in literature (Table 1).

**b. Steam treated straw**

Straw is an abundant agricultural by-product consisting of the dry stalks of cereal crops and accounts for approximately half of the crop yield; making it a particularly suitable feedstock for AD (Croce et al., 2016). Due to its lignocellulosic nature pre-treatment methods such as steam are often applied to weaken the structures and increase degradability. Wheat straw is generally composed of 44.3% lignin, 24.5% cellulose and 22.3% hemicellulose (Croce et al., 2016). Utilising steam pre-treatment has been reported to increase biogas and $CH_4$ yields by altering the chemical composition (Bauer et al., 2014; Theuretzbacher et al., 2015). In this study, the only samples to produce biogas yields significantly higher than untreated straw were pre-treated at 4 and 8 bar and produced approximately 35 and 31%, respectively, more biogas than untreated straw which is similar to other studies that found an increase from using steam pre-treatment (Amon et al., 2007).These yields obtained were in accordance to the range of values reported in literature (Table 1). The slight differences in yields could be attributed to a temperature difference during pre-treatment or possibly the pressure the steam was injected at. Ferreira et al., (2014) found a 19 to 24% increase in $CH_4$ yield when using steam treated straw in the temperature range of 170 to 220°C which is higher compared to this study (Amon et al., 2007). The higher biogas yields suggest steam pre-treatment increases the biodegradability of lignocellulosic feedstocks. In contrast, straw treated at 6 bar generated almost 4% less biogas than untreated straw and straw treated at 10 bar only produced approximately 9% more than untreated straw. Theuretzbacher et al., (2015) conducted a study using similar temperatures to this study (140 to 178°C) and concluded that steam treated straw produced similar or lower biogas yields compared to untreated straw (Theuretzbacher et al., 2015). Although pressure was not reported in Theuretzbacher et al., (2015) study, it was stated the "Economizer SE" was used for pre-treatment which operates between 5 and 8 bar; allowing the assumption that the same pressure was applied to the samples tested. Similarly, their research found the highest $CH_4$ yield to be from the sample treated at 140°C which is in accordance to results from this study. Other studies have reported that pre-treatment temperature has the greatest impact on biomass composition and hence $CH_4$ yield (Theuretzbacher et al., 2015). Untreated straw experienced a lag in biogas production until day 5 where it reached a cumulative gas production rate like the pre-treated substrates. The initial lag period indicates steam pre-treatment increases the digestibility of lignocellulosic biomass by breaking down its complex structures increasing its accessibility to bacteria (Theuretzbacher et al., 2015). The yields observed in this study indicate that higher yields are obtained at lower pressure and temperature (4 bar and 140°C) and when increasing pre-treatment pressure and temperature there is an optimal range to remain within to avoid producing yields lower or similar to untreated straw. These variations in findings challenge the economic feasibility of steam pre-treatment of wheat straw when optimum conditions are not used. As a result, depending on the pre-treatment intensity (temperature and pressure), the pre-treated straw can result in lower yields (Theuretzbacher et al., 2015).

### c. Livestock slurries

CL and chick litter both produced higher biogas yields and at a faster rate than cow manure which could be due to their contrasting composition. Niu et al., (2014) reported that CL has a high protein content of 25% TS which is one of the largest contributions to anaerobic fermentation (Niu et al., 2014). In comparison, cow manure has a low protein and lipid content and approximately one-third of its total organic matter is comprised of fibre (Niu et al., 2014). It is probable that the cow manure evaluated in this study also consisted of a high fibre content which prompted its low yield and slow degradation. It has been noted that CL has a higher percentage of readily biodegradable material than cow manure which could explain the difference in initial cumulative biogas production rates(Kelleher et al., 2002). In relation to literature, the $CH_4$ potential of cow manure (76±20 mL/g $VS_{feedstock}$) was lower than most values (Table 1). It is plausible that the cow's diet (potato starch, wood chips and onion waste) is responsible for the lower yield. Studies have noted that manure characteristics and biogas potentials are reliant on a series of factors: animal species, diet, protein and fibre content, age, housing and more (Kelleher et al., 2002). Costa et al., (2013) found that cows fed with diets richer in concentrate (protein mixed with maize silage) as opposed to forage had lower levels of fibre and resulted in higher biogas yields in AD. Besides, livestock manure as a feedstock for AD can be precarious as it contains a mixture of feathers or hair, spilled feed, bedding and mortality, which leads to a great variability in reported biogas and $CH_4$ yields from previous research (Table 1).

### d. Agro-industrial waste

The biogas production of fish waste exhibited a calculated lag phase of 3.7 days which can be attributed to the high lipid and protein content in fish waste which takes longer for bacteria to degrade but in turn produces high yields (Costa et al., 2013). Nges et al., (2012) estimated that nearly 64 million tonnes of fish waste is generated annually worldwide, mostly being underutilised as low-value animal feed (Sosa-Hernandez et al., 2016), which evidences the potential for renewable energy formation if treated by AD. Rendered fat washings similarly consists mostly of protein (37%) and lipids (53%) (Hejnfelt and Angelidaki, 2009; Pitk et al., 2012) The measured biogas yield of rendered fat washings was high (1379±125 mL/g $VS_{feedstock}$) but corresponds to values presented in literature (Table 1). The biogas yield from potato waste (768±27 mL/g $VS_{feedstock}$) was slightly higher than values in previous studies but the $CH_4$ content (51.7%) corresponds with reported values (50.8%) (Kryvoruchko et al., 2009). Potato waste generated the fourth highest biogas yield of all substrates tested; a possible explanation for this is its C/N ratio. The C/N ratio of potato waste is approximately 26 which falls within the optimal ratio for biogas production (Kryvoruchko et al., 2009; Mu et al., 2017).

**e. Fruit and vegetable waste**

Potato peels comprised a relatively low $CH_4$ content (52.3%) producing an ultimate $CH_4$ yield of 331±2.5 mL/g $VS_{feedstock}$. This could be explained by its high starch composition which is easily converted to VFAs and $CO_2$ but not further to $CH_4$ (Mu et al., 2017). Previous literature reports $CH_4$ yields of 239 to 390 mL/g $VS_{feedstock}$ (Table 41). This study has observed a similar yield in a decreased digestion time of 20 days (Liang and McDonald, 2015; Parawira et al., 2005). Onion waste, BPs and avocado seed exhibited a similar biogas production pattern where gas rapidly increased in the initial days before slowing down around day 8. A possible explanation is the more easily degraded compounds were consumed in the first 8 days and slow degradation of more complex compounds followed after (Parawira et al., 2004). The biogas yields produced from BP (568±17 mL/g $VS_{feedstock}$) and onion waste (576±15 mL/g $VS_{feedstock}$) correspond with values reported in literature (Table 1). To the best of our knowledge, no studies have been reported for avocado seed as a feedstock for AD, which could constitute a viable source of renewable energy as avocado processing generates a significant amount of waste; with approximately 18,144 tonnes of avocado residues generated in 2008 for Mexico alone (Siddiq et al., 2012).

**f. Co-digestion**

Co-digestion is often recommended when dealing with animal manure to enhance biogas yields, decrease ammonia content and improve the C/N ratio (Akyol et al., 2015). Chicken litter is a high ammonia substrate, with C/N ratios typically around 9 – 13 (Zahan et al., 2018), hence why many studies have been performed co-digesting CL with substrates such as, lignocellulosic biomass, various animal manures, FW and fruit and vegetable wastes. Conversely, to the best of our knowledge no studies have been reported co-digesting CL with banana waste or avocado waste. Both mixtures tested containing ACL reached their ultimate biogas potential on day 19 as compared to day 5 for BP/WBCL due to the high lipid content in avocado pulp which takes longer for bacteria to degrade. In comparison, overripe WBs and BPs are comprised of 16 and 33% monosaccharides, respectively; making them easily degradable at a faster rate (lag phase: 2.2 and 1.9 days, respectively) (Table 3) (Emaga et al., 2011). It is probable the banana mixtures generated higher $CH_4$ content compared to avocado pulp mixtures (Table 3) due to their high sugar content which a portion is transformed directly to acetic acid and made available to acetate-utilising methanogens. It is likely to be the reason why 50:50-WBCL (422±46 mL/g $VS_{feedstock}$) generated a higher $CH_4$ yield than 50:50-ACL (393±62 mL/g $VS_{feedstock}$). However, it was observed that the co-digested substrate with the least amount of CL (80:20-ACL) produced the highest $CH_4$ yield (517±13 mL/g $VS_{feedstock}$). It is to be remarked that the biogas production of 80:20-ACL and 50:50-ACL was 84% and 54% higher than the expected from their calculated individual contribution as single substrates, suggesting a synergistic effect this co-digestion mixture.

## 5. Conclusions

All energy crops and agricultural by-products tested can be successfully anaerobically digested to produce biogas. The highest biogas yield (1379 ± 125 mL/g $VS_{feedstock}$) was obtained from digestion of rendered fat washings. Steam pre-treatment of wheat straw does not guarantee higher biogas yields in comparison to untreated wheat straw, indicating that it may not be economically attractive if pre-treatment conditions are not optimised. Synergistic benefits of co-digesting CL with fruit wastes were evidenced. Co-digesting CL with 50% and 80% avocado pulp led to a 54% and 84% higher biogas production, respectively, than expected from contribution of the separate substrates.

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## 7. Figure captions

- Fig. 1 - Cumulative biogas yield for a) energy crops, b) untreated and steam pre-treated wheat straw, c) livestock slurries, d) agro-industrial wastes, e) fruit and vegetable wastes and f) co-digested substrates.
- Table 1 – Comparison of biogas and methane yields from literature for substrates tested in BMP tests.
- Table 2 – Characteristics of substrates tested prior to anaerobic digestion tests.
- Table 3 – Biogas and methane yields with calculated lag phases using the modified Gompertz model for all substrates

**Table 1 –** Comparison of biogas and methane yields from literature for substrates tested in BMP tests.

| Feedstock | Biogas yield | Methane yield | Digestion time | Temperature | TS | VS | Reference |
|---|---|---|---|---|---|---|---|
| | mL/g $VS_{added}$ | mL/g $VS_{added}$ | days | °C | % w/w | % w/w | |
| **Energy crops** | | | | | | | |
| Ryegrass | 431 | 249 | 25 | 38 | 15.2 | 13.2 | Vitez et al. 2015 |
| | 654 | 399 | 25 | 38 | 25.1 | 21.9 | |
| | 621 | 318 | 35 | 37 | 35.4 | 33.3 | Hubner et al. 2011 |
| Grass | 520 | 267 | 30 | 39 | 91.2 | 85.2 | Dandikas et al. 2014 |
| | 370 | 226 | 70 | 55 | 18.9 | 15.3 | Poulsen and Adelard 2016 |
| Triticale (whole crop) | 650 | 329 | 30 | 39 | 91.2 | 85.3 | Dandikas et al. 2014 |
| | 681 | 388.0 | 28 | 35 | 33.7 | 32.0 | Heiermann et al. 2009 |
| Rye (whole crop) | 614 | 356 | 28 | 35 | 29.3 | 27.4 | Heiermann et al. 2009 |
| | 668 | 336 | 30 | 39 | 91.6 | 86.4 | Dandikas et al. 2014 |
| Wheat (whole crop) | 679 | 360 | 40 | 35 | 36.3 | 34.7 | Rinco et al. 2012 |
| **Steam pre-treated straw** | | | | | | | |
| Untreated straw | - | 245 | 50 | 35 | 89.5 | 82.1 | Ferreira et al. 2014 |
| Pre-treated 170°C | - | 291 | 50 | 35 | 89.5 | 82.1 | |
| Pre-treated 200°C | - | 304 | 50 | 35 | 89.5 | 82.1 | |
| Untreated hay | 420 | 234 | 30 | 37.5 | 87.1 | 82.0 | Bauer et al. 2014 |
| Pre-treated 170°C | 469 | 281 | 30 | 37.5 | 35.1 | 32.6 | |
| Untreated wheat straw | 613 | 276 | 40 | 37.5 | 89.6 | 85.4 | Theuretzbacher et al. 2015 |
| Pre-treated 140°C | 550 | 275 | 40 | 37.5 | 24.0 | 23.0 | |
| Pre-treated 160°C | 555 | 261 | 40 | 37.5 | 22.1 | 21.3 | |
| Pre-treated 178°C | 563 | 270 | 40 | 37.5 | 14.6 | 14.0 | |
| **Livestock slurries** | | | | | | | |
| Chicken litter | 496 | 291 | 28 | 37 | 24.9 | 19.4 | Li et al. 2013 |
| | 311 | 127 | 30 | 35 | 26.8 | 16.7 | Wang et al. 2012 |
| | 529 | 282 | 60 | 35 | 28.8 | 18.8 | Zhang et al. 2014 |
| Cow manure | - | 204 | 20 | 35 | 6.5 | 5.3 | Lehtomaki et al. 2007 |
| | 276 | 149 | 40 | 37 | 12.4 | 11.2 | Akyol et al. 2015 |
| **Agro-industrial wastes** | | | | | | | |
| Rendered fat washings | - | 978 | 36 | 37.5 | 99.0 | 100 | Pitk et al. 2012 |
| | - | 562 | 35 | 55 | 99.4 | 99.2 | Hejnfelt and Angelidaki 2009 |
| Fish waste | 1104 | 828 | 33 | 37 | 41.2 | 35.5 | Ivo et al. 2012 |
| | 672 | 390 | 29 | 27 | 32.2 | 17.8 | Mshandete et al. 2004 |
| Cheese whey | 270 | 174 | 9 | 32 | 3.0 | 2.7 | Antonelli et al. 2016 |
| | 732 | 450 | 8 | 37.5 | 4.5 | 4.0 | Skripsts et al. 2011 |
| Waste yeast | 604 | 284 | 20 | 37 | 8.2 | 8.1 | Sosa-Hernandez et al. 2016 |
| Spent brewer's grain | 770 | 445 | 24 | 38.5 | 93.3 | 86.7 | Dido et al. 2014 |
| | 660 | 389 | 67 | 37 | 21.1 | 20.1 | Bochmann et al. 2015 |
| | 386 | 229 | 8 | 37 | 25.0 | 23.4 | Panjicko et al. 2015 |
| Sugar beet tops | - | 520 | 60 | 37 | 11.0 | 9.2 | Parawira et al. 2004 |
| | - | 280 | 25 | 55 | 11.0 | 8.5 | Fang et al. 2011 |
| Potato waste | 654 | 332 | 38 | 37.5 | 16.4 | 15.0 | Kryvoruchko et al. 2009 |
| **Fruit and vegetable waste** | | | | | | | |
| Banana peels | 535 | 251 | 12 | 37 | 16.5 | 14.8 | Nathoa et al. 2014 |
| | 327 | 180 | 25 | 37 | 10.7 | 9.3 | Bardiya et al. 1996 |
| | - | 210 | 23 | 38 | 14.6 | 14.1 | Clarke et al. 2008 |
| Potato peels | 373 | 239 | 40 | 35 | 9.2 | 7.5 | Liang and McDonald 2015 |
| | 557 | 390 | 50 | 37 | 19 | 18.1 | Parawira et al. 2005 |
| Onion waste | 560 | 342 | 14 | 35 | 92.6 | 89.0 | Romano and Zhang 2011 |

**Table 2 –** Characteristics of substrates tested prior to anaerobic digestion tests.

| Substrate | TS % w/w | VS % w/w | VS/TS % w/w | Origin |
|---|---|---|---|---|
| **Energy crops** | | | | |
| Ryegrass | 35.18 ± 0.01 | 33.98 ± 0.01 | 96.59 ± 0.00 | Silage field in SE England |
| Grass | 27.08 ± 0.00 | 25.01 ± 0.00 | 92.36 ± 0.00 | |
| Triticale (whole crop) | 26.54 ± 0.00 | 24.89 ± 0.00 | 93.78 ± 0.00 | Crop trial plots in East England |
| Soya (whole crop) | 14.13 ± 0.00 | 11.73 ± 0.00 | 83.01 ± 0.00 | |
| Rye (whole crop) | 39.59 ± 0.01 | 37.91 ± 0.01 | 95.76 ± 0.00 | |
| Wheat (whole crop) | 38.20 ± 0.00 | 36.83 ± 0.00 | 96.41 ± 0.00 | |
| **Pre-treated straw** | | | | |
| Untreated straw | 49.56 ± 0.03 | 47.11 ± 0.03 | 95.06 ± 0.00 | Pilot plant in North Wales |
| Pre-treated 4 bar | 37.82 ± 0.02 | 35.39 ± 0.02 | 93.57 ± 0.00 | |
| Pre-treated 6 bar | 74.74 ± 0.00 | 69.06 ± 0.00 | 92.40 ± 0.00 | |
| Pre-treated 8 bar | 49.04 ± 0.01 | 45.85 ± 0.01 | 93.50 ± 0.00 | |
| Pre-treated 10 bar | 42.03 ± 0.01 | 39.49 ± 0.01 | 93.96 ± 0.00 | |
| **Livestock slurries** | | | | |
| Chicken litter | 38.10 ± 0.02 | 13.48 ± 0.00 | 35.38 ± 0.03 | Free-range egg farm in SE England |
| Chick litter | 33.97 ± 0.00 | 29.42 ± 0.00 | 86.61 ± 0.00 | Farm in SE England |
| Cow manure | 30.63 ± 0.01 | 24.21 ± 0.01 | 79.04 ± 0.01 | |
| **Agro-industrial wastes** | | | | |
| Rendered fat washings | 0.28 ± 0.00 | 0.20 ± 0.00 | 71.43 ± 0.05 | Rendering facility in SE England |
| Fish waste | 15.22 ± 0.00 | 13.73 ± 0.00 | 90.21 ± 0.00 | Fish processing plant in SE England |
| Cheese whey | 6.32 ± 0.00 | 5.79 ± 0.00 | 91.61 ± 0.00 | Cheese farm in Norfolk, England |
| Waste yeast | 8.11 ± 0.00 | 7.24 ± 0.00 | 89.27 ± 0.00 | Brewery in Waterbeach, England |
| Spent grain | 47.13 ± 0.01 | 44.95 ± 0.01 | 95.37 ± 0.00 | |
| Sugar beet tops | 13.35 ± 0.00 | 11.32 ± 0.00 | 84.79 ± 0.01 | Crop trial plots in East England |
| Potato waste | 33.92 ± 0.00 | 32.16 ± 0.00 | 94.81 ± 0.00 | Farm in SE England |
| **Fruit and vegetable waste** | | | | |
| Banana peels | 9.12 ± 0.00 | 7.77 ± 0.00 | 85.20 ± 0.00 | Local supermarket in Cambridge, England |
| Potato peels | 21.45 ± 0.01 | 20.26 ± 0.01 | 94.45 ± 0.00 | |
| Avocado seed | 43.99 ± 0.00 | 42.40 ± 0.00 | 96.39 ± 0.00 | |
| Onion waste | 13.71 ± 0.00 | 12.86 ± 0.00 | 93.80 ± 0.00 | Farm in SE England |
| **Co-digestion** | | | | |
| 80% Avocado 20% Chicken litter | 27.03 ± 0.00 | 20.28 ± 0.00 | 75.03 ± 0.01 | Market in London and a free-range egg farm in Southeast England |
| 50% Avocado 50% Chicken litter | 29.50 ± 0.00 | 16.99 ± 0.00 | 57.59 ± 0.00 | |
| 50% Banana peels 50% Chicken litter | 24.08 ± 0.00 | 11.67 ± 0.00 | 48.46 ± 0.00 | |
| 50% Whole banana 50% Chicken litter | 26.11 ± 0.00 | 13.00 ± 0.00 | 49.79 ± 0.01 | |
| **Inoculum for batch digestion** | | | | |
| Inoculum | 4.75 ± 0.91 | 3.09 ± 0.96 | 65.05 ± 6.15 | Full-scale AD plant in Southwest England |
| Diluted inoculum (Rendered fat) | 0.37 ± 0.00 | 0.29 ± 0.00 | 78.38 ± 0.02 | |
| Diluted inoculum (Cheese whey) | 1.47 ± 0.00 | 0.96 ± 0.00 | 65.31 ± 0.01 | |

Note: The inoculum was diluted when testing rendered fat and cheese whey in order to maintain the required inoculum to substrate VS ratio while requiring a lower amount of feedstock sample, which was limited in these cases.

**Table 3 –** Biogas and methane yields with calculated lag phases using the modified Gompertz model for all substrates

| Substrate | Biogas yield mL/g $VS_{added}$ | | Methane yield mL/g $VS_{added}$ | | Methane content % | λ days | $R^2$ |
|---|---|---|---|---|---|---|---|
| **Energy crops** | | | | | | | |
| Ryegrass | 481 | ± 34.0 | 294 | ± 20.8 | 61.1 | 2.57 | 1.000 |
| Grass | 622 | ± 127.4 | 359 | ± 73.5 | 57.7 | 0.00 | 0.996 |
| Triticale (whole crop) | 510 | ± 18.0 | 338 | ± 11.9 | 66.3 | 6.80 | 1.000 |
| Soya (whole crop) | 594 | ± 30.7 | 376 | ± 19.4 | 63.3 | 1.79 | 1.000 |
| Rye (whole crop) | 432 | ± 18.7 | | - | - | 3.50 | 1.000 |
| Wheat (whole crop) | 404 | ± 38.2 | 283 | ± 26.8 | 70.1 | 5.61 | 1.000 |
| **Pre-treated straw** | | | | | | | |
| Untreated straw | 326 | ± 22.3 | 197 | ± 13.5 | 60.4 | 4.86 | 1.000 |
| Pre-treated 4 bar | 441 | ± 41.5 | 271 | ± 25.5 | 61.5 | 3.11 | 1.000 |
| Pre-treated 6 bar | 313 | ± 17.0 | 198 | ± 10.8 | 63.2 | 2.02 | 1.000 |
| Pre-treated 8 bar | 426 | ± 6.6 | 262 | ± 4.1 | 61.5 | 2.97 | 1.000 |
| Pre-treated 10 bar | 356 | ± 10.6 | 217 | ± 6.5 | 61.0 | 2.87 | 1.000 |
| **Livestock slurries** | | | | | | | |
| Chicken litter | 307 | ± 24.3 | 184 | ± 14.0 | 59.9 | 2.18 | 1.000 |
| Chick litter | 371 | ± 23.3 | 229 | ± 11.5 | 61.8 | 1.62 | 1.000 |
| Cow manure | 123 | ± 32.3 | 76 | ± 19.9 | 61.6 | 2.89 | 1.000 |
| **Agro-industrial wastes** | | | | | | | |
| Rendered fat washings | 1379 | ± 125.3 | | - | - | 5.27 | 1.000 |
| Fish waste | 898 | ± 107.0 | 545 | ± 65.0 | 60.7 | 3.69 | 1.000 |
| Cheese whey | 818 | ± 9.9 | 359 | ± 4.4 | 43.9 | 3.51 | 1.000 |
| Waste yeast | 412 | ± 3.3 | 220 | ± 1.8 | 53.3 | 0.00 | 1.000 |
| Spent grain | 644 | ± 42.1 | 390 | ± 25.5 | 60.5 | 2.50 | 1.000 |
| Sugar beet tops | 686 | ± 26.3 | 449 | ± 45.8 | 65.5 | 1.16 | 1.000 |
| Potato waste | 768 | ± 27.3 | 397 | ± 14.1 | 51.7 | 6.21 | 1.000 |
| **Fruit and vegetable waste** | | | | | | | |
| Banana peels | 568 | ± 17.4 | | - | - | 4.27 | 1.000 |
| Potato peels | 633 | ± 7.0 | 331 | ± 2.5 | 52.3 | 3.66 | 1.000 |
| Avocado seed | 474 | ± 23.1 | | - | - | 2.21 | 1.000 |
| Onion waste | 576 | ± 15.2 | 360 | ± 9.5 | 62.5 | 6.53 | 1.000 |
| **Co-digestion** | | | | | | | |
| 80% Avocado 20% Chicken litter | 811 | ± 19.1 | 517 | ± 12.5 | 63.8 | 3.85 | 1.000 |
| 50% Avocado 50% Chicken litter | 602 | ± 93.7 | 393 | ± 62.0 | 65.3 | 4.62 | 1.000 |
| 50% Banana peels 50% Chicken litter | 425 | ± 15.9 | 283 | ± 10.6 | 66.6 | 1.87 | 1.000 |
| 50% Whole banana 50% Chicken litter | 625 | ± 2.8 | 422 | ± 46.0 | 67.5 | 2.16 | 1.000 |

The termination criteria for ultimate biogas and $CH_4$ yield was when daily biogas production was less than 5%. 20 days was the longest time for a substrate to reach the criteria.

**Highlights**

1. The biomethane potential of 29 different organic substrates was evaluated.
2. Highest biogas recorded for rendered fat washings (1379 ± 125 mL/g $VS_{feedstock}$).
3. Synergistic benefits of co-digesting CL with fruit wastes were evidenced.
4. Co-digestion of chicken litter with avocado pulp led to synergistic biogas yields.

**Conflicts of interest**

I declare that there are no conflicts of interest to declare for manuscript BITEB-D-18-00198

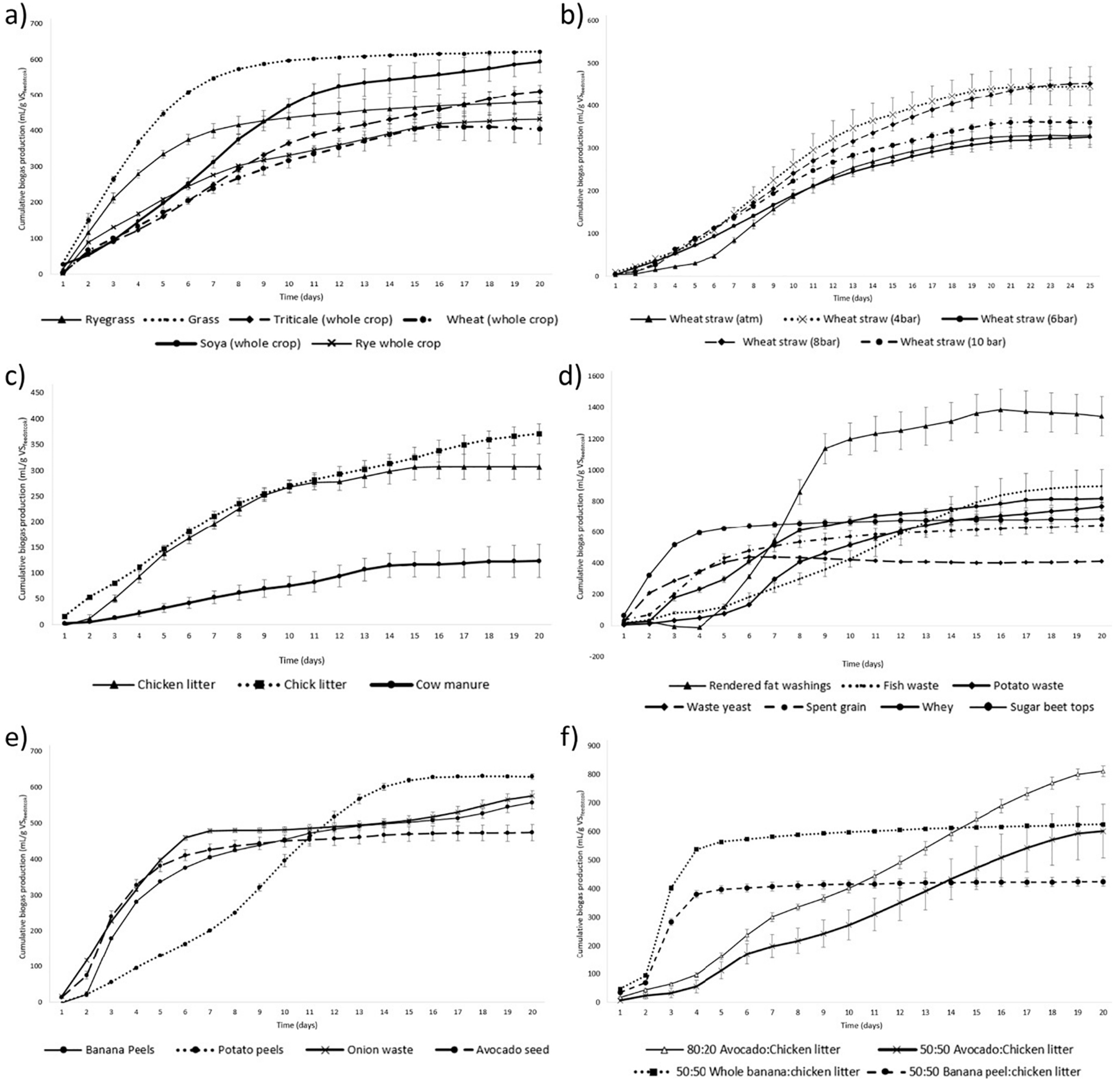
a)
Cumulative biogas production (mL/g VS feedstock)
Time (days)
Ryegrass
Grass
Triticale (whole crop)
Wheat (whole crop)
Soya (whole crop)
Rye whole crop
b)
Cumulative biogas production (mL/g VS feedstock)
Time (days)
Wheat straw (atm)
Wheat straw (4bar)
Wheat straw (6bar)
Wheat straw (8bar)
Wheat straw (10 bar)
c)
Cumulative biogas production (mL/g VS feedstock)
Time (days)
Chicken litter
Chick litter
Cow manure
d)
Cumulative biogas production (mL/g VS feedstock)
Time (days)
Rendered fat washings
Fish waste
Potato waste
Waste yeast
Spent grain
Whey
Sugar beet tops
e)
Cumulative biogas production (mL/g VS feedstock)
Time (days)
Banana Peels
Potato peels
Onion waste
Avocado seed
f)
Cumulative biogas production (mL/g VS feedstock)
Time (days)
80:20 Avocado:Chicken litter
50:50 Avocado:Chicken litter
50:50 Whole banana:chicken litter
50:50 Banana peel:chicken litter

Figure 1

# Evaluation of anaerobic digestibility of energy crops and agricultural by-products

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2018-11-16



Spence A, Madrigal EB, Patil R, Bajón Fernández Y. (2019) Evaluation of anaerobic digestibility of energy crops and agricultural by-products. Bioresource Technology Reports, Volume 5, February 2019, pp. 243-250

https://doi.org/10.1016/j.biteb.2018.11.004