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What is the effect of mandatory pasteurisation on the biogas transformation of solid slaughterhouse wastes?



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ABSTRACT

The effect of mandatory pasteurisation on Category 3 offals, according to the Animal By-Products Regulation (ABPR 1069/2009/EC), was determined using Biochemical Methane Potential (BMP) assays as well as kinetic and statistical analysis. Pasteurised and unpasteurised offals sampled from cattle, pig and chicken slaughterhouses were characterised and their specific methane yields (SMYs) and their bioavailability was assessed. The resultant SMYs were high ($465\text{--}650\text{ mLCH}_4\text{ gVS}^{-1}$) with no statistically significant increase in methane production identified due to pasteurisation. However, the kinetics of the biogas transformation processes highlighted increased bioavailability of the organics due to pasteurisation. This was brought to light by the change in maximum daily SMY from day 22 to day 1 for the cattle offal ($p = 0.001$), day 17 to day 1 for chicken offal ($p = 0.025$) and an increase of 18.8% in the maximum daily SMY of the pig offal on day 1 ($p = 0.003$). The increased bioavailability of the offals manifested itself in two ways with the determining factor being identified as the physical characteristics of the fats i.e. particle size. Firstly reducing the hydrolytic lag phase for the cattle offal, $\lambda = 7.46\text{--}1.52$ days ($p = 0.013$). Secondly, causing increased accumulation of Long Chain Fatty Acids to acute inhibitory levels in the chicken and pig offal indicated by increased lag phases $\lambda = 5.05\text{--}21.91$ days ($p = 0.012$), $\lambda = 15.54\text{--}23.04$ days ($p = 0.007$) respectively.

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1. Introduction

The slaughtering industry is a major facet of the agri-food sector in Ireland. More than 1.6, 2.9 and 84.8 million cattle, pigs and chickens respectively are slaughtered annually in Ireland (Central Statistics Office, 2012). Approximately 46%, 26% and 32% (cattle, pig and chicken) of the total animal weight slaughtered is not used for food consumption and is considered process by-products of varying value (Central Statistics Office, 2012; Edström et al., 2003; Verheijen et al., 1996). Traditionally these waste streams were treated through the rendering process (Bayer et al., 2012). The enforcement of the ABPR in 2002 (1774/2002/EC repealed by 1069/2009/EC) to prevent the outbreak and spread of disease, dictated the need for higher hygiene regulations, tighter process controls and exclusion of the use of some animal by-products in traditional uses (European Commission, 2009). The implementation of these regulations reduced the economic value of these materials for rendering and in many cases they have to be disposed of through incineration. The regulations regard biogas transformation as a suitable treatment method for a variety of animal

by-products, provided approved pre-treatments are applied (European Commission, 2005; Palatsi et al., 2011). Legislation dictates slaughterhouse wastes must be treated by different thermal pre-treatments prior to use in biogas transformation according to its category (European Commission, 2009). Three categories are defined (Kirchmayer et al., 2003; Braun and Kirchmayer, 2003; Hejnfelt and Angelidaki, 2009):

- Category 1, high risk material (material presenting the highest risk of containing animal diseases), is not permitted to be treated through biogas transformation under any circumstances.
- Category 2, high risk animal by-products (perished animals and/or animals slaughtered but not intended for human consumption), must be sterilised to $133\text{ }^{\circ}\text{C}$ under 3 bars for 20 mins.
- Category 3, low risk material (meat containing wastes from food industry and slaughterhouse waste of animals fit for human consumption), must be treated to a minimum of $70\text{ }^{\circ}\text{C}$ for 60 mins.

Slaughterhouse waste streams are considered as model substrates for biogas transformation due to their high fat and protein content. However, they are also regarded as difficult substrates for the very same reasoning, primarily the high fat content (Palatsi

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et al., 2011). The simplified biogas transformation of fats is outlined in Fig. 1. This process depends on the syntrophic nature of the acetogenic and methanogenic bacterial populations (Palatsi et al., 2011). The hydrolysis rates of fats are dependent both on their chemical characteristics as well as physical characteristics such as the available surface area (particle size). Sayed et al. (1988) found the liquefaction of fats to be rate limiting in slaughterhouse wastewater when high amounts of suspended solids were present due to their low bioavailability as a result of their lower surface area and insolubility. The most common reasons for the instability of the biogas transformation process, especially with regards to the treatment of substrates with a high fat content, are the production of inhibitory compounds known as intermediate fermenters such as Volatile Fatty Acids (VFAs) and Long Chain Fatty Acids (LCFAs), produced during acidogenesis and acetogenesis (Palatsi et al., 2011). Palatsi et al. (2010, 2011) along with Cirne et al. (2007) observed rapid accumulation of VFAs during the initial stages of the biogas transformation of substrates with high fat contents, indicating that the hydrolytic-acidogenic bacteria did not inhibit the substrate degradation and that the process was held at the acetogenic and methanogenic stages, shaded grey in Fig. 1. LCFAs can only be degraded through syntrophic interactions of acetogenic and methanogenic bacterial communities and as such the inhibition of the acetogenesis stage results in methane production reducing or ceasing during the initial lag phase of LCFAs acetogenesis (Bayer et al., 2012; Palatsi et al., 2011; Cirne et al., 2007; Sousa et al., 2007, 2009). The inhibitory effect of LCFAs is a recoverable phenomenon, related to the physical adsorption of LCFA which can hinder the solubility of the substrate through microbial cell walls along with the slow growth rate of LCFA consuming bacteria (Palatsi et al., 2011; Hejnfelt and Angelidaki, 2009; Salminen and Rintala, 2002). Consequently, when LCFA inhibition occurs it can be monitored as an initial delay in methane production or as a long lag phase before complete degradation of the substrate occurs (Palatsi et al., 2011; Hejnfelt and Angelidaki, 2009).

The pasteurisation of offals prior to use as a substrate for biogas transformation is required to meet regulations set out by the

European Commission to avoid potential risks to humans and animals under the ABPR. However it could potentially influence higher performance of the biogas transformation process also, in terms of the bioavailability of organics increasing methane production rate as well as increasing Specific Methane Yield (SMY). In terms of raising performance, the goal of the pre-treatment is to make the components of the waste stream more bioavailable, which means that the proteins and fats of the waste stream are more readily available to the bacterial populations thus reducing the hydrolysis period. However increasing bioavailability may also have negative connotations; by increasing the rate at which intermediate fermenters are produced, inhibition may occur through rapid accumulation of compounds such as LCFAs and VFAs. Conflicting effects of thermal pre-treatments such as sterilisation (133 °C and 3 bars for 20 min) or the more common pasteurisation (70 °C for 60 min), on the methane yield of slaughterhouse wastes have been reported.

Edström et al. (2003) compared the potential gas yield from Pasteurised (P) and Un-pasteurised (UP) mixtures of slaughterhouse waste, food waste and liquid manure. They concluded that the P mixture resulted in a fourfold increase in biogas production in comparison to the UP mixture. The biogas yield increased from 0.311 Lbiogas gVS⁻¹ to 1.14 Lbiogas gVS⁻¹. Hejnfelt and Angelidaki (2009) investigated the effects of both sterilisation and pasteurisation on the methane yield from mixed pork waste and reported that neither pre-treatments had an effect on achieved methane yields. Cueto et al. (2010) assessed the effects of sterilisation on the methane yield of poultry slaughterhouse waste and its co-digestion with the organic fraction of municipal solid waste (OFMSW). The attempt of increasing methane yield by means of application of sterilisation was not successful for either mixes tested due to the instability of the digesters. The methane yields observed were in fact reduced after pre-treatment was applied, with a reduction of between 10% and 34%. Rodríguez-Abalde et al. (2011) evaluated effects of thermal pre-treatments on the methane yield of two solid slaughterhouse wastes, poultry and pig slaughterhouse by-products. Pasteurisation was applied to both wastes and the pig waste stream was also sterilised to observe the effects. Varied results were reported; pasteurisation and sterilisation had a significant effect on the methane yield of the pig waste, over 50% increase for both pre-treatment methods. This increment was not observed with the chicken waste with only a 2.6% increase observed indicating pre-treatment had no significant benefits to the process.

It is clear that the effect of thermal pre-treatment on the methane yield of slaughterhouse waste is extremely varied and a categorical statement as to the increase or decrease on the methane/biogas yield cannot be made. The focus of this work is to study the anaerobic biodegradability and methane potential of solid slaughterhouse waste streams, under standardised conditions, in order to determine the effect of mandatory pasteurisation imposed by the ABPR.

2. Materials and methods

2.1. Substrates

Solid slaughterhouse wastes were gathered from cattle, pig and chicken slaughtering facilities. The selected solid waste fractions were Category 3 soft offals produced during the evisceration process. The general consistency of the offals in their sampled state was as a heterogeneous solid waste, consisting of large identifiable individual components of animal entrails and fat trimmings. Both the chicken and pig offal contained the digestive tract contents of the animals. The digestive tract contents of the cattle are removed

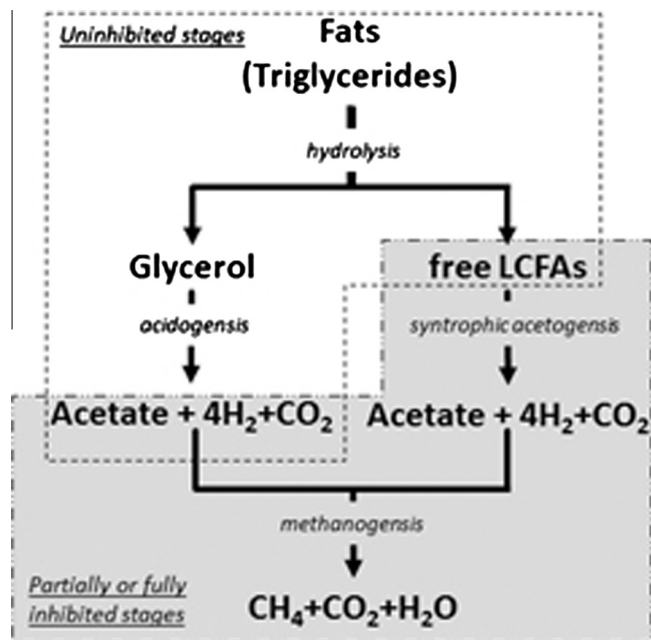


Fig. 1. Biodegradation stages of fats to biogas outlining stages affected by high levels of LCFA accumulation, derived from Palatsi et al. (2010, 2011) and Cirne et al. (2007).

prior to evisceration of the cattle and are treated as a separate waste stream. The highly controlled environment in which these wastes are produced significantly reduced the probability of interferents and pollutants entering the waste streams.

2.1.1. Sampling

The primary sample was provided by the slaughtering facilities grounded on providing typical representation of offal from the slaughtering of a single head in the case of cattle and pig and multiple heads in the case of chickens. The consistency of the primary samples of the offals did not permit their direct use in accurate BMP assays or composition analysis. As such the entire primary samples were firstly macerated in order to reduce the particle size (<8 mm) and then blended using a high rate paddle mixer until maximum “homogeneity” was achieved. This preparation process improved the homogeneity of the primary samples considerably. However it is important to note that even after the preparation process the offals are still characterised as heterogeneous, which needs to be taken into account when considering the preparation of secondary samples for reference analyses and BMP testing as well as when considering the results (Bayer et al., 2012). Three dimensional sampling of the prepared primary sample was carried out in order to ensure representative secondary sampling of the substrates.

2.2. Inoculum

LCFA toxicity has been shown to vary with the type of anaerobic inoculum utilised and was more correlated to the inoculum's physical characteristics (specific surface area and size distribution) than to their biological characteristics (Hwu et al., 1996; Chen et al., 2008). Suspended and flocculated inoculums, which have a higher specific surface area, have been shown to suffer from a much greater degree of LCFA inhibition than granular inoculum (Hwu et al., 1996; Chen et al., 2008). Accordingly, the BMP assays carried out in this study employed granular mesophilic inoculum, due to the high possibility of LCFA inhibition, sourced from a mesophilic Up-flow Anaerobic Sludge Blanket (UASB) reactor treating dairy processing waste. The inoculum was harvested directly from the reactor sampling ports and thus contained residual biodegradable organic material. Consequently the inoculum was “degassed” i.e. pre-incubated, in order to reduce the residual biodegradable organic material in the inoculum and limit the background gas produced during the testing. The degassing of the inoculum was carried out under the same temperature range as the operational temperature of the inoculum source and the experimental set-up. No additional external nutrients were added to the inoculum, it was assumed that basic nutrient requirements for biogas transformation were provided by the inoculum.

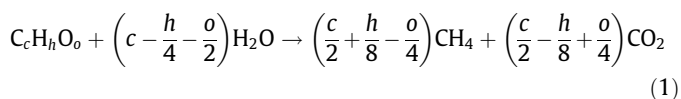
2.3. Analytical methods

The composition analysis was carried out in terms of basic, organic and elemental characterisation. The basic parameters used for substrate and inoculum description were the Total Solids (TS) and Volatile Solids (VS) content determined in accordance to standard methods (U.S. Environmental Protection Agency, 2001). The organics (VS) were further broken down into primary constituents of fats, proteins and carbohydrates. Fats and proteins were determined by an approved laboratory for the microbiological testing of ABP in accordance with Commission Regulation 142/2011/EU implementing the ABPR (European Commission, 2009, 2011). The difference between VS, fats and protein content was designated as carbohydrates. The elemental composition (C, H, N) was determined following the standard operating procedure of a CE440

Elemental Analyser, with O being designated as the difference between VS and the C, H and N content.

2.4. Ultimate methane yield estimation

The Ultimate Methane Yield (UMY) of the offals was calculated based on the stoichiometry of methane production and the empirical formula of the organic waste streams, Eq. (1). Commonly referred to as the Buswell formula (Buswell and Mueller, 1952)



The above equation in most cases will be optimistic since neither non-degradable organics nor energy demand of the bacterial populations is considered.

2.5. Experimental methods

The biogas transformation of the substrates was performed in batch mode utilising BMP assays. The assays were maintained under strict anaerobic conditions within a mesophilic temperature range. The methane potential of the substrates was evaluated based on their SMY defined as the total volume of methane produced during the incubation period per amount of substrate (organic fraction) initially added, measured as mLCH₄ gVS⁻¹. The effect of the P was evaluated utilising a combination of the SMY as well as analysis of the BMP production curves (Section 2.6.1) and the kinetics of the methane production determined through mathematic modelling (Section 2.6.2).

2.5.1. BMP assays

The BMP procedure employed in this study was based on the principles described by DIN 38 414 (S8) (1985) and VDI 4630 (2006) with alterations to the gas measurement system (Section 2.5.2) for direct measurement of the methane fraction of the biogas produced. A graphical representation of the experimental setup is provided in Fig. 2. Known amounts of substrate and degassed inoculum, using an inoculum to substrate ratio of 2 based on VS content, were digested in 1000 mL Duran bottles (working volume of 900 mL). Triplicate assays for each waste stream were performed to allow for the heterogeneity of the substrates. Triplicate assays containing only degassed inoculum were also implemented as control reactors to correct for background gas produced. All reactors were statically incubated, within water baths at 36–39 °C, until substrate exhaustion. Mixing was provided on a daily basis prior to recording the methane production. A guideline for the termination of the test was when the daily gas production was equivalent to approximately 1% of the total volume produced over the period of the test. The initial incubation period selected for this study was 30 days as the majority of the biodegradation would be completed at this stage (Labatut et al., 2011). If the gas production towards day 30 was observed to be larger than 1% of the total volume produced the incubation period was extended to 50 days to allow complete degradation of the substrate.

2.5.2. Gas measurement system

The methane produced was determined directly through positive liquid displacement. To directly measure the methane fraction of the biogas produced the biogas was passed through an alkaline solution (0.5 M NaOH), removing the carbon dioxide fraction and the need for daily offline gas composition analysis. To ensure that saturation of the alkaline solution did not occur the produced gas was periodically tested using a GA5000 handheld biogas analyser to ensure the absence of carbon dioxide from the measured gas.

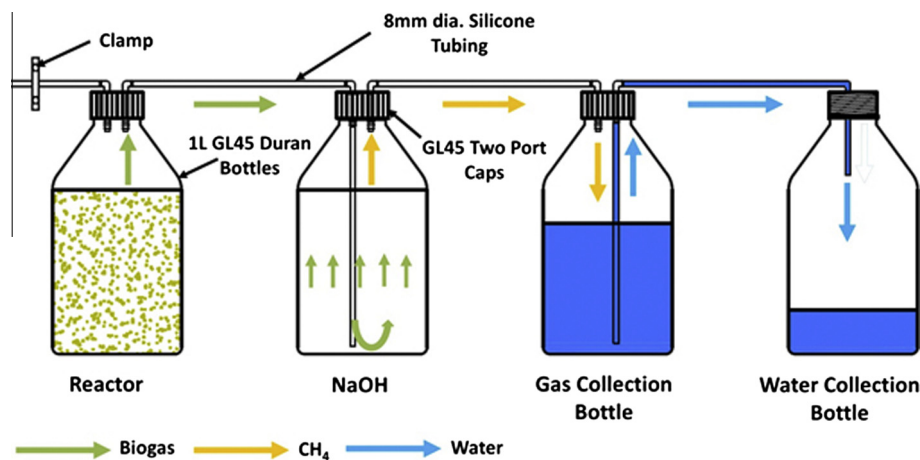


Fig. 2. Graphical representation of experimental setup.

The methane production was measured daily to allow the kinetics of the process to be followed and to provide direction as to the stability of the process. At the end of the incubation period, a pH measurement was taken of all BMP assays to ensure that the methane production had not ceased due to acidification or if alkaline solution had been drawn into the reactors due to negative pressure.

2.5.3. Pasteurisation

The offals were tested in both UP and P forms to determine the influence of the pasteurisation process on biodegradability and methane potential. The pasteurisation was carried out in accordance to ABPR for Category 3 wastes prior to use in biogas transformation (70 °C for a minimum of 60 mins) (European Commission, 2009; Kirchmayer et al., 2003). The pasteurisation had a definite effect on the physical characteristics of the offals from visual inspection. The rendering of the fats from the chicken and pig offal, resulted in the reduction in size of the offal particles and a more “liquid” appearance of the sample. After cooling the consistency was a very malleable paste. While the fats in the cattle offal, which could be differentiated from the rest of the offal as off-white spheres prior to pasteurisation, appeared to melt. Once cooled the fats in the cattle offal returned to a solid form and again were clearly discernible from the other offal components as was the case pre-pasteurisation. It was assumed that the pasteurisation process would not alter the VS content, organic or elemental composition as the operational temperature of 70 °C and duration of the process is too low to reduce the organic content of the samples substantially, nor can this process add any organics to the original sample. That was not to say that the process did not alter the chemical structure of the organics, i.e. promoting the splitting of complex fats into simpler and more bioavailable constituents to promote shorter hydrolysis periods. The chemical alteration of the organics is not as easily appraised as the physical alterations observed and must be assessed through the evaluation of the results of the BMP assays.

2.6. Evaluation of BMP assays

2.6.1. BMP production curves

A primary output of BMP assays includes cumulative SMY curves which can follow a diverse range of patterns. The patterns these curves follow are far from trivial and have meaningful implications. The kinetics of the different stages of the biogas transformation process and ultimately the shape of the methane production curves is primarily controlled by the biodegradability characteristics of the substrate and the production of inhibitory

intermediate fermenters and performance of the methanogenic bacterial populations. Labatut et al. (2011) outlined their relevance in aiding in identifying important biodegradability characteristics of the substrate and any inhibition issues.

2.6.2. Kinetic modelling

Mathematic modelling of microbial growth has been used extensively to estimate various parameters in relation to microbial growth curves. In this study the modified form of the Richards sigmoidal function by Zwietering et al. (1990), Eq. (2), was applied to the experimental data to determine the maximum methane production potential (A), maximum rate of methane production (μ_m) and the duration of the lag phase (λ). The modified Richards model also incorporates a fourth parameter (v) that permits flexibility in the shape of the curve, fundamental when dealing with the possibility of partial inhibition due to intermediate fermenters.

$$y = A \left\{ 1 + v \cdot \exp(1 + v) \cdot \exp \left[\frac{\mu_m}{A} \cdot (1 + v)^{\left(1 + \frac{1}{v}\right)} \cdot (\lambda - t) \right] \right\}^{-1/v} \quad (2)$$

λ is an indication of the minimum time taken for the methanogenic bacteria to acclimate to the environment and is defined as the x-axis intercept of the tangent of the inflection point of the curve (Zwietering et al., 1990; Atlas, 2009). It can be used as an indicator of the degree of inhibition (larger λ), if any, or an increase in bioavailability (smaller λ) during the incubation period. A nonlinear least square regression analysis was performed using SPSS (IBM SPSS 22) to determine A , μ_m , λ and v . At the same time the standard error and coefficient of determination or correlation coefficient (R^2) was obtained to determine the correlation of the modelled and experimental data.

2.7. Statistical analysis

All of the experiments were performed in triplicate, and the results are expressed as mean values and relative standard deviations where applicable. All statistical analysis was carried out using SPSS. Normal distribution was assumed based on the agreement of parametric and non-parametric testing and thus all inference was carried out using t -tests and a level of significance (α) of 0.05.

3. Results and discussions

3.1. Substrate composition analysis

The characterisations of the individual offals are summarised in Table 1. The organic analysis of the offals indicated that the VS

Table 1

Characterisation of sampled offals, all determinations performed in triplicated with relative standard deviation applied where relevant.

	TS (%)	VS (%)	Fat (%TS)	Protein (%TS)	Carbohydrate (%TS)	C:H:O:N (%TS)
<i>Untreated offal</i>						
Cattle offal (UP)	65.2 ± 1.79	98.6 ± 0.08	58.1	26.5	14.02	65.8:10.8:18.9:3.1
Chicken offal (UP)	40.3 ± 3.69	96.6 ± 0.70	36.1	34.0	26.5	55.3:9.4:26.7:5.1
Pig offal (UP)	27.9 ± 0.22	95.2 ± 0.02	41.8	31.6	21.83	59.6:9.0:22.0:4.6
<i>Treated offals</i>						
Cattle offal (P)	53.9 ± 3.24	98.6 ± 0.08	58.1	26.5	14.02	65.8:10.8:18.9:3.1
Chicken offal (P)	44.9 ± 2.17	96.6 ± 0.70	36.1	34.0	26.5	55.3:9.4:26.7:5.1
Pig offal (P)	33.1 ± 2.76	95.2 ± 0.02	41.8	31.6	21.83	59.6:9.0:22.0:4.6

were composed of primarily fats, followed by protein and finally carbohydrates as the lowest portion. A significant difference in the carbohydrate contents of the pig and chicken offal was seen in comparison to the cattle offal. This can be attributed to the digestive tract contents remaining in these waste streams during the slaughtering process. The high fat content of the offals raises concern in terms of possible inhibition through the accumulation of intermediate fermenters from the hydrolysis of the fats.

3.2. BMP results

The results of the BMP assays are summarised in Table 2, presenting both the SMY and Gross Methane Yield (GMY) along with the methane based degradability (SMY/UMY) of the P and UP assays. Graphical representations of both daily and cumulative SMY are shown in Fig. 3, for the P and UP offals.

3.2.1. Unpasteurised offals

The biogas transformation and methane yield of the offals in their UP state was initially investigated. All three UP offals presented with high SMYs, ranging from 515.47 to 465.34 mLCH₄ gVS⁻¹ as expected due to the high fat and protein content. The cumulative SMY curves observed (shown in Fig. 3) were of an elongated S-shape, characterised by a high rate of methane production in the initial phase of the incubation period, followed by a reduced daily methane yield, leading to a significant increase in methane production and finally reaching a plateau to the maximum methane yield. The elongated S-shape curve is a typical curve associated with organic waste streams with high concentrations of complex compounds in particular fats.

The slower degradation of the fats resulted in a hydrolytic lag phase observed for all of the untreated offals, indicated by the slight reduction in daily SMY for a period of time. This identified the hydrolysis of the fats as the rate limiting step in the biogas transformation of the UP offals. The continued degradation of the

glycerol and LCFAs into soluble compounds for methanogenic bacteria meant that during the hydrolytic lag phase a steady methane production was observed. If the accumulation of LCFAs became too high the methane production would have been reduced by a larger degree to more or less zero indicating inhibition of the syntrophic acetogenesis and methanogenesis stages.

LCFAs are surface active compounds and in aqueous systems behave like synthetic surfactants (Salminen and Rintala, 2002). Therefore a small floating scum layer on the surface of the reactors was observed on all of the BMP assays for the UP offals. As methane production was seen to increase the floating scum layer could be seen to reduce, due to the degradation (acetogenesis) of the LCFAs. The methane production patterns were similar for the UP offals with variations in the length of the lag phase caused by the delayed hydrolysis of the fats, as evident from the daily methane production patterns shown in Fig. 3. The severity of the floating scum layers also varied, proportionally in relation to the length of the lag phase, for each of the UP offals studied.

The maximum average daily SMY for UP cattle offal occurred on day 23 (33.9 ± 3.86 mLCH₄ gVS⁻¹ d⁻¹). This was due to the rate limiting step; hydrolysis of the fats. The hydrolytic lag phase can be clearly seen from day 4 until 11 where methane production is at a steady daily yield and subsequently rapidly increases as LCFAs are broken down during the period of 11–23 days. The maximum daily SMY transpired to be the latest of the UP offals, indicating that the hydrolysis of the fats was at a slower rate than that of the UP pig and chicken offals. This was most likely due to the limited available surface area of the fats for the hydrolytic bacteria to act on in comparison to the other offals i.e. the fats existed in larger particle sizes therefore less surface for bacteria to act on resulting in slower rate of degradation, liquefaction of fats was rate limiting (Sayed et al., 1988). A much smaller hydrolytic lag phase was observed for the UP chicken offal, 4–5 days observed from day 1–6, with methane production promptly increasing after day 6 impending towards a maximum average daily SMY on day 17

Table 2

Results of BMP assays, relative standard deviation applied where necessary.

	IP ^a	SMY (mLCH ₄ gVS ⁻¹)	GMY (mLCH ₄ g ⁻¹)	VS degradation (%)	UMY (mLCH ₄ gVS ⁻¹)	SMY/UMY (%)
<i>Untreated offals</i>						
Cattle offal (UP)	30	515.47 ± 58.8	331.30 ± 37.8	89.06 ± 4.2	889.6	57.9 ± 6.6
Chicken offal (UP)	30	499.11 ± 35.4	194.32 ± 13.8	88.70 ± 5.0	751.8	66.4 ± 4.7
Pig offal (UP)	50	465.34 ± 62.4	123.77 ± 16.6	95.73 ± 0.6	807.3	57.6 ± 7.7
<i>Treated offals</i>						
Cattle offal (P)	30	569.11 ± 43.8	302.93 ± 23.3	N.d.	889.6	64.0 ± 4.9
Cattle offal (P)	50	650.92 ± 46.9	346.48 ± 24.9	99.99 ± 1.37	889.6	73.2 ± 5.3
Chicken offal (P)	50	501.13 ± 40.6	217.36 ± 17.6	100* (116.47 ± 4.6)	751.8	66.7 ± 5.4
Pig offal (P)	50	518.18 ± 38.3	163.43 ± 12.1	100* (118.62 ± 2.5)	807.3	64.2 ± 4.8

UP – Unpasteurised, substrate with no pre-treatment applied.

P – Pasteurised, substrate treated according to ABP regulations for Category 3 offals (70 °C for min 1 hour), VS content, organic and elemental characteristics assumed unchanged due to pasteurisation.

N.d. – not determined due to BMP still running so sampling could not take place.

^a IP – Incubation period in days, duration of BMP.

* VS destruction shown as; 100* (calculated VS degradation ± standard deviation).

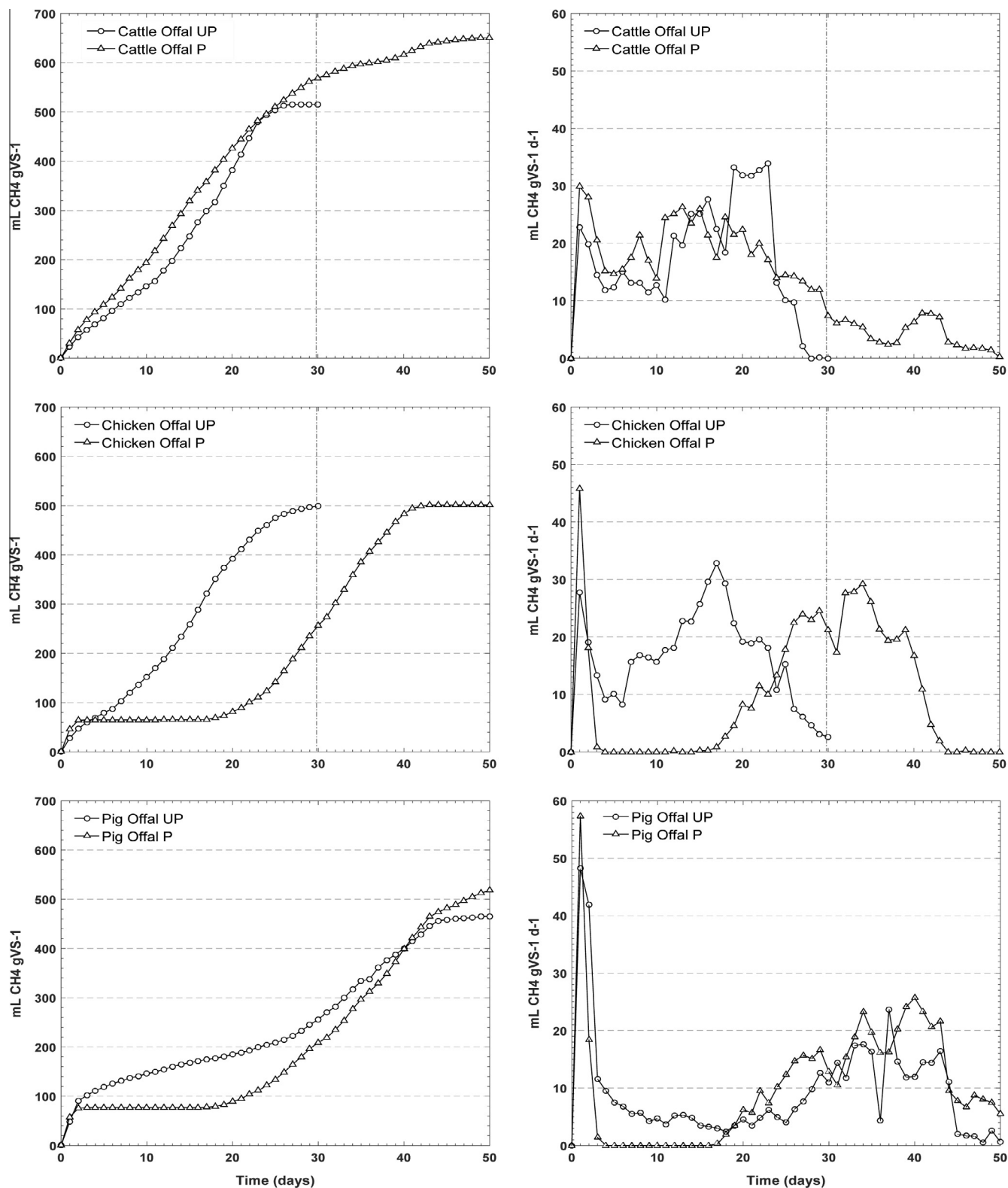


Fig. 3. Daily and cumulative SMYs for offals both pasteurised and unpasteurised.

($32.8 \pm 0.39 \text{ mLCH}_4 \text{ gVS}^{-1} \text{ d}^{-1}$). The earlier incidence of the maximum daily SMY can be attributed to the fats existing in a more bioavailable form (primarily a smaller particle size).

Evident differences were seen in the biogas transformation of the UP pig offal. Firstly the average maximum daily SMY ($48.2 \pm 1.45 \text{ mLCH}_4 \text{ gVS}^{-1} \text{ d}^{-1}$) occurred at a much earlier time,

day 1 of the incubation period. This was followed by a much more dramatic reduction in methane production over the next two days (suggesting process instability). Secondly the lag phase observed (day 5–25), identified from the daily methane production graph (Fig. 3), was substantially longer than previously observed for the UP cattle and chicken offals. These two manifestations are related.

The significantly higher maximum daily SMY at a much earlier stage indicates that the organics present in the pig offal were in a more bioavailable form and thus easily hydrolysed, resulting in more glycerol and LCFAs produced at an earlier stage in the incubation period. The glycerol then fermented into VFA, alcohols and other acids and consumed by the methanogenic bacteria responsible for the increased methane yield on day 1. The production of LCFAs was at a higher rate, resulting in a higher accumulation than seen in UP chicken and cattle offal causing an acute toxic effect on the syntrophic acidogenic bacteria (reducing efficiency of methane production for a small period of time). Thus, reducing the methane production and extending the lag phase due to the larger amount of LCFAs present. The longer lag phase resulted in the methane production being greater than 1% of the overall methane production by day 30, thus the incubation period was extended to 50 days. It is worth noting that the accumulation of the LCFAs although partially impeding the syntrophic activity of the acetogenic and methanogenic bacteria was not classed as outright inhibition rather the process being in an inhibited steady state. A condition where the process is running stably but less efficiently and lower methane production due to the acute toxic effect of a substance, in this case LCFAs (Chen et al., 2008). The inhibited steady state resulted in the elongated S-shape cumulative SMY curve being drawn out in comparison to those observed for UP cattle and chicken offal.

3.2.2. Pasteurised offals

The biogas transformation and methane yield of the offals in a P state was investigated under identical process conditions as for the UP assays. Again all three P offals presented with high SMYs, ranging from 501.13 to 650.92 mLCH₄ gVS⁻¹ over a 50 day incubation period. The kinetics of the biogas transformation of the P offals differed from what was observed for the UP assays. The elongated S-shapes of the cumulative specific methane curves (Fig. 3) were replaced by steeped curves for P pig and chicken offal while the P cattle offal was more representative of a reverse L-shape curve. The incubation period for all of the P offals was extended to 50 days as per the stipulation outlined in Section 2.5.1.

The P cattle offal presented an average maximum daily SMY of 29.9 mLCH₄ gVS⁻¹ d⁻¹ ± 15.8% on day 1 of the incubation period while maintaining ample methane production within the first 11 days, observed as a hydrolytic lag phase for the UP samples. This materialised in the cumulative specific methane production curve of the P cattle offal having a steeper slope in the initial phase of the incubation period resulting in a reverse L-shape curve. Of the three P offals studied the cumulative curves of the cattle offal differed from those observed for the P pig and chicken offals (stepped curves). The stepped curve typically indicates that some form of inhibition occurred within the reactors, raising the question as to why this was not observed for the treated cattle offal considering

it had the highest fat content (53.9%); suggesting that it would be the most vulnerable to inhibition. Again the larger particle size of the fats as outlined previously for UP cattle offal can be seen to have an effect here (Sayed et al., 1988). The larger particle size of fats in the cattle offal resulted in a lower surface area for the hydrolytic bacteria to act on reducing the rate of accumulation of LCFA and thus avoiding inhibition.

The P chicken and pig offal presented maximum average daily SMYs (45.8 mLCH₄ gVS⁻¹ d⁻¹ ± 0.31% and 57.3 mLCH₄ gVS⁻¹ d⁻¹ ± 3.49% respectively) on day 1 of the incubation period also. The maximum daily SMYs occurring at this stage was an indication of an increased bioavailability and thus rate of hydrolysis and consequently a more rapid build-up of LCFA and other intermediate fermenters which manifested as a rapid decrease in the daily SMY to zero over a period of 48 h. This was caused by the inhibition of the acetogenic and methanogenic bacterial populations, due to the rapid accumulation of high concentrations of LCFAs. In literature LCFA inhibition has been demonstrated as a reversible phenomenon (Palatsi et al., 2011). This is echoed in the results obtained in this study; after a period of approximately 15 days the methane production was seen to slowly increase indicating the recovery of the bacterial populations (acetogenic and methanogenic) within the P chicken and pig offal assays. In tandem the floating scum layers were seen to break down indicating the degradation of the LCFAs.

3.3. Kinetics of methane production

Table 3 summarises the results of the kinetic study carried out using the modified Richards equation. To evaluate the soundness of the model results, the predicted methane yields were plotted against the experimental values as shown in Fig. 4. All of the models provided reasonably good fits from visual inspection, while the R² values were in the range of 0.989–0.999. The largest difference between experimental and modelled SMYs was less than 3%, and less than 2% in most cases. Patil et al. (2012) reported errors of up to 8.7% when predicting methane yields from water hyacinth using sigmoidal growth curves. Thus the modified Richards models were reasoned as a good fit for the biogas transformation of both P and UP offals.

3.4. Effects of pasteurisation

3.4.1. Increasing methane yield

For both P and UP assays cattle offal presented the highest SMY over the 30 and 50 day incubation periods. It is important to note as the incubation period of the UP cattle offal was only for 30 days and considering the similarity of the kinetics of the biogas transformation, the direct comparison of the results at 50 days against the 30 day results would be inaccurate in this particular case. Over a

Table 3
Kinetic parameters estimated by the modified Richards model.

	A (mLCH ₄ gVS ⁻¹)	μ _m (mLCH ₄ gVS ⁻¹ d ⁻¹)	λ (days)	v	R ²	Difference between V ₀ and A
<i>Untreated offals</i>						
Cattle offal (UP)	523.39	32.03	7.80	4.59	0.997694	+1.53%
Chicken offal (UP)	512.28	27.12	5.24	1.96	0.999369	2.64%
Pig offal (UP)	462.33	16.07	15.66	89.4	0.999069	−0.64%
<i>Treated offals</i>						
Cattle offal (P)	659.31	23.51	1.46	0.23	0.999235	+1.29%
Chicken offal (P)	501.67	28.61	21.93	7.78	0.991109	+0.11%
Pig offal (P)	510.01	23.13	23.08	9.90	0.989448	−1.58%

UP – Unpasteurised, substrate with no pre-treatment applied.

P – Pasteurised, substrate treated according to ABP regulations for Category 3 offals (70 °C for min 1hour), VS content, organic and elemental characteristics assumed unchanged due to pasteurisation.

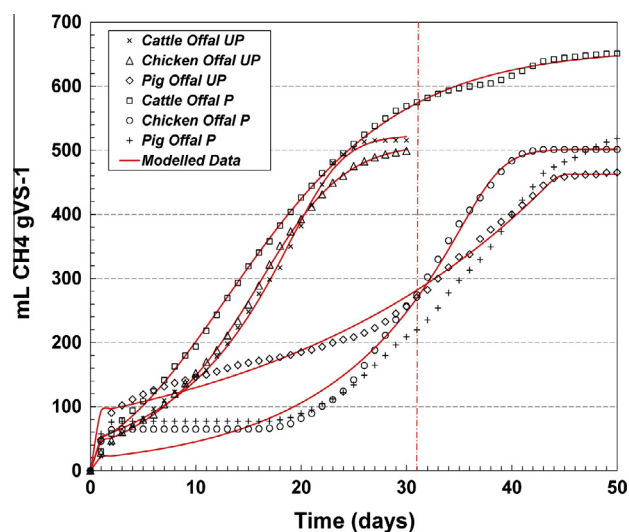


Fig. 4. Cumulative specific methane yield and modified Richards model plots for P and UP offals.

30 day incubation period the SMY was seen to increase from 515.47 to 569.11 mLCH₄ gVS⁻¹, a 10.4% increase due to pasteurisation. This increase in the SMY was found to be statistically insignificant ($p = 0.459$). The treating of the LCFA inhibition in the P chicken offal as an initial delay in methane production or long lag phase before completed degradation of the substrate, as reported by a number of other studies (Hejnfelt and Angelidaki, 2009; Palatsi et al., 2010; Pereira et al., 2005), was asserted through the removal of the 15 day lag phase from the cumulative SMY curve over a 50 day incubation period. This resulted in an elongated S-shape curve comparable ($R^2 = 0.996$) to that of the UP chicken offal from 30 days of incubation, as seen in Fig. 5. As a result the direct comparison of the SMYs from the 30 day incubation period for the UP chicken offal and the 50 day incubation period for the P chicken offal was deemed acceptable. The chicken offal showed a 0.4% increase in the SMY, 499.11–501.13 mLCH₄ gVS⁻¹, as a result of the pasteurisation which was proven to be statistically insignificant ($p = 0.634$).

The accumulation of LCFAs from the hydrolysis of the fats in the UP pig offal caused an inhibited steady state to occur, where the process was running stably but less efficiently and with lower methane production due to the acute toxic effect of the LCFA (Chen et al., 2008). This inhibited steady state lasted for approximately 25 days. In the case of the P pig offal the increased rate of hydrolysis and the rapid accumulation of LCFAs, resulted in a fully inhibited state, however the duration of this period was less at 15 days of zero methane production. Consequently the two states of inhibition were deemed comparable; UP pig offal low methane yield over longer period, P pig offal zero methane yield over shorter period making the SMYs over the two 50 day incubation periods comparable. The pasteurisation caused an 11.4% increase in the SMY of the pig offal. This increase from 465.34 to 518.18 mLCH₄ gVS⁻¹ was determined to be statistically insignificant ($p = 0.455$).

3.4.2. Increasing bioavailability of organics

Pasteurisation failed to have a significant effect on the SMYs of the offals however the pasteurisation process did have an effect on the bioavailability of the offals. This was distinguished initially through the variation of the cumulative SMY curves of all three P offals. The change in shape of the cumulative SMY curves (Fig. 3) is a direct result of the change in rate of the biodegradation of the organics, primarily the bioavailability of the substrate to

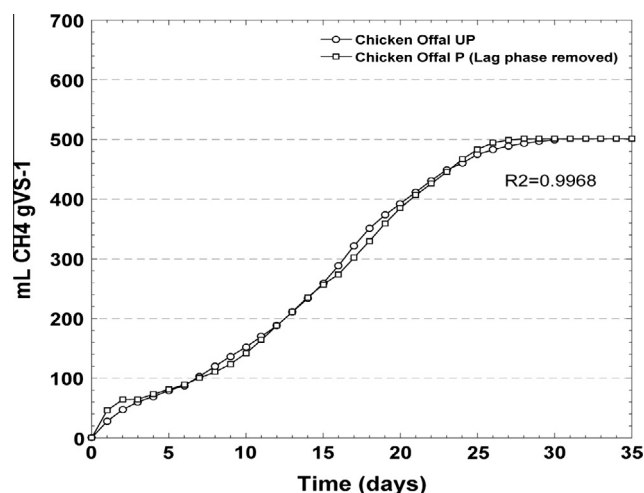


Fig. 5. Comparison of cumulative specific methane curves for UP chicken and P chicken after removal of 15 day lag phase observed between day 4 and 19.

hydrolytic bacteria. Further analysis of the BMP results revealed the effect of the pasteurisation on the bioavailability of the organics.

The average maximum daily SMY was seen to shift from day 22 for UP cattle offal to day 1 for P cattle offal which was deemed as a significant change ($p = 0.001$). Granted the maximum specific daily methane yield was 11.8% lower at 29.9 mLCH₄ gVS⁻¹ d⁻¹ ± 15.8%, there was a statistically significant increase ($p = 0.012$) of 28.5% in methane production observed within the first 15 days of incubation of the treated cattle offal. This indicated a shift to the greater part of the methane production occurring in the first 15 days of the incubation period rather than the latter half of the incubation period. This materialised in the cumulative methane curve of the treated cattle offal having a steeper slope in the initial phase of the incubation period altering the curve to a reverse L-shape rather than an elongated S-shape for the untreated cattle offal. The increased bioavailability was also demonstrated by the reduction in λ estimated from the kinetic modelling, 7.80 days to 1.46 days, a statistically significant decrease ($p = 0.013$) highlighting the increased rate of hydrolysis.

The effect of pasteurisation on chicken offal in terms of bioavailability was also apparent from the daily SMY trends observed. The maximum daily SMY of the P chicken offal was seen to occur 16 days earlier (day 17–1) when compared to that of the UP chicken offal, a statistically significant shift ($p = 0.025$). An average 39.6% increase in maximum daily SMY was also observed across the triplicate assays for the P chicken offal, a proven statistically significant increase ($p = 0.001$). This was a clear indication that the bioavailability of the fats increased by the pasteurisation process; consequently they were hydrolysed at a higher rate resulting in higher methane production at an earlier stage. The increased bioavailability was most likely due to the rendering action during pasteurisation releasing the fats from the meat and tissue of the offal. The same phenomenon was observed in the P pig offal with a large increase in maximum daily SMY occurring on day 1. The increase of 18.8% (48.2–57.3 mLCH₄ gVS⁻¹ d⁻¹) was seen to be statistically significant ($p = 0.003$) again confirming the increased bioavailability of the organics. These results indicated that the pasteurisation did in fact promote the splitting of the complex fats and perhaps some of the proteins into simpler and more bioavailable forms resulting in earlier and increased maximum daily SMY. However for P chicken and pig offal this resulted in a rapid accumulation of inhibitory intermediate fermenters causing inhibition of the methanogenic bacteria.

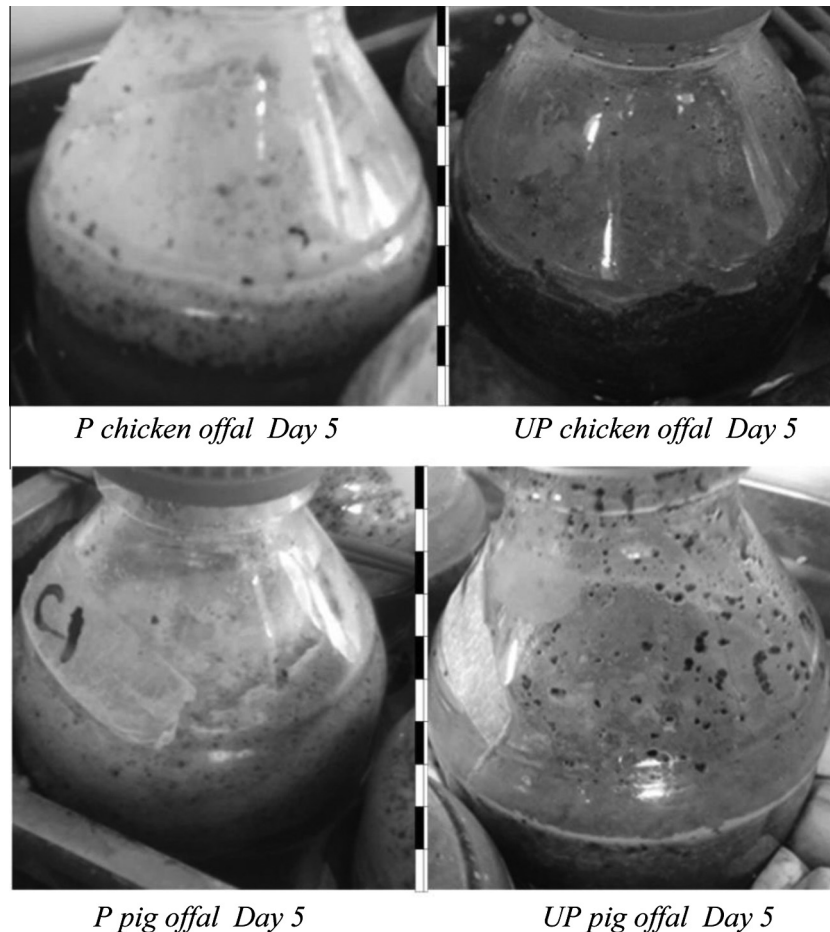


Fig. 6. Floating fat/scum layer after of P and UP chicken and pig offal after 5 days of incubation.

3.4.3. LCFA accumulation

An increase in λ was seen for both P chicken and pig offal, 5.24–21.93 days and 15.66–23.08 days respectively, both statistically significant results ($p = 0.012, 0.007$) suggesting some form of inhibition/instability in the assays. Larger λ and ceasing of methane production observed for the P chicken and pig offals indicated the occurrence of something other than a delay in hydrolysis. Inhibition of the methanogenic bacteria was assumed as the methane production in both sets of assays dropped to zero for extended periods. As the reactors were sealed for the duration of the BMP's the identification of the concentration of intermediate fermenters during the incubation period could not be quantified. However one of the properties of LCFAs is that they are surface active compounds and in aqueous systems will act as synthetic surfactants (Salminen and Rintala, 2002). This results in thick floating layers occurring if a large accumulation of LCFAs occurs. For both the P pig and chicken offal substantial large floating scum layers in comparison to the UP offals were observed after a period of approximately 5 days (beginning of inhibition period) as can be seen in Fig. 6 by the white foaming. This was a clear indication that a large accumulation of LCFA had occurred resulting in the ceasing of methane production from day 4 onwards in both cases. Therefore the rate limiting factor in these cases was attributed to LCFA accumulation and inhibition of the acetogenic and methanogenic bacterial populations, rather than substrate recalcitrance to the hydrolytic bacterial population as was the case in the UP assays. The rapid accumulation of LCFAs in comparison to the UP pig and chicken offal assays is also an indication of the increased

bioavailability of the organic compounds within the P assays as mentioned earlier.

The LCFAs were gradually broken down, signified by the reduction of the foaming layers as the assays reached their respective λ values. This was accompanied by a revival of methane production in all assays signifying the recovery of the methanogenic bacterial population, leading to the exponential phase of the curve followed by the stationary phase where the asymptote (max SMY) was eventually reached. The recovery of the bacterial populations within the assays after LCFA inhibition demonstrated that it is partial in nature and the possibility of bacterial populations to acclimate to higher levels of inhibitory compounds for fat rich waste streams.

4. Conclusions

The BMP assays performed on characterised slaughterhouse offals showed high biodegradability and SMY; but that fat content, particle size as well as pasteurisation had an effect of the process kinetics as a whole. The pasteurisation process increased the bioavailability of the organics, primarily fats, which demonstrated both positive and negative connotations; increased initial daily SMY coupled with a reduced hydrolytic lag phase (cattle offal) as well as promoting more rapid accumulation of intermediate fermenters (chicken and pig offal) causing LCFA inhibition. Despite the recorded inhibition after pasteurisation the process was able to fully recover syntrophic methanogenic activity and fully degrade the pasteurised offals.

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