

## Use of diluted poultry manure as a low-cost emulsifier for anaerobic digestion of used cooking oil

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### Abstract

The aim of this study was to evaluate the anaerobic co-digestion of diluted poultry manure (DPM) and used cooking oil (UCO). Mixtures of DPM with different UCO dosages (1 to 6% v/v) were prepared using a high-shear emulsifier and digested in batch anaerobic reactors. Increasing the UCO dosage increased the emulsion COD (from initially 64 to 182 g · L<sup>-1</sup>) but emulsion stability was affected adversely. UCO at the optimum dosage (1.5 to 2.0% v/v) was further digested in a semi-continuous mesophilic anaerobic reactor, to assess process feasibility at organic loading rates (OLRs) up to 8 g · L<sup>-1</sup> · d<sup>-1</sup>. The reactor was stable, yielding biogas at 0.45 L · g<sup>-1</sup> COD, with low supernatant COD (<6 g · L<sup>-1</sup>), negligible VFA accumulation and no foaming.

**Key words:** anaerobic digestion, biogas, fat oil and grease, high shear emulsification, poultry manure, pre-treatment

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### INTRODUCTION

Edible oil and fat consumption is increasing worldwide, and is expected to reach 178 Mt · a<sup>-1</sup> in 2025 (OECD/FAO 2016). Hotels, restaurants, cafés and residences are the main contributors. In Europe, as a whole, edible vegetable oil use per capita averages 15 kg · a<sup>-1</sup>, and up to 26 kg · a<sup>-1</sup> around the Mediterranean (Lin *et al.* 2013).

Used cooking oil (UCO) is important mainly due to its high organic content. At household level, about 20% of the UCO is discharged to drains (Wallace *et al.* 2017), the remainder going to landfill and contributing to greenhouse gas emissions (GHG), leachate generation and odor release (Chen *et al.* 2012; Shen *et al.* 2013). UCO can clog sewerage pipelines severely, and increase the costs of sewage management and treatment. Large quantities of UCO are generated by hotels, restaurants and cafes, and are often used for biodiesel production or direct combustion (Long *et al.* 2012). Finally, due to its high methane potential (Labatut *et al.* 2011), UCO can be used as a co-substrate in anaerobic digesters, to increase biogas yield and methane recovery (Alqaralleh *et al.* 2016).

In the European Union around 113 Mt · a<sup>-1</sup> of poultry manure are generated (Foged *et al.* 2012). These by-products are traditionally applied to land as organic- and nitrogen-rich fertilizer (Thangarajan *et al.* 2013). Uncontrolled land disposal can result in environmental consequences, however, such as GHG emissions (NH<sub>3</sub>, NO<sub>x</sub>, N<sub>2</sub>O), release of odors and pathogens, and eutrophication of water bodies (ten Hoeve *et al.* 2014). Anaerobic digestion of poultry manure results in waste stabilization, volume reduction, and odor control, combined with the production of methane gas that can be used as a source of energy (Sakar *et al.* 2009; Rodriguez-Verde *et al.* 2018). It is often considered a problematic substrate for mono-digestion, however, due to its high protein and ammoniacal nitrogen contents (Elasri & Elamin 2016). Anaerobic co-digestion with lipid-rich wastes can thus increase the carbon to nitrogen ratio and improve the anaerobic digestion process.

The aim of this study was to evaluate the anaerobic co-digestion of diluted poultry manure (DPM) and UCO. The substrates were pre-treated using a high-shear emulsifier to disintegrate the UCO and promote its solubilization. Such mechanical pre-treatment can also decrease the lipid particle size, and increase the surface area to volume ratio and thus the availability of the substrate to microbes (Harris & McCabe 2015; Carrere *et al.* 2016). Mixtures of DPM with different UCO dosages were digested in batch anaerobic reactors, while the optimum mixture was further treated in a semi-continuous anaerobic reactor, to assess process feasibility (biogas production rate, methane yield, effluent quality, VFA accumulation and degree of foaming).

## MATERIALS AND METHODS

### Substrates origin and characteristics

The poultry manure (without bedding material) originated from an egg producing facility (Ioannina Region, Greece). It was characterized by total solids (TS) =  $332 \text{ g} \cdot \text{kg}^{-1}$  and volatile solids (VS) =  $289 \text{ g} \cdot \text{kg}^{-1}$ . The UCO was obtained from a local restaurant. Its characteristics were – density =  $0.907 \text{ g} \cdot \text{mL}^{-1}$ , acidity =  $9.7 \text{ mg-NaOH} \cdot \text{g}^{-1}$ , saponification value =  $90 \text{ mg-HCl} \cdot \text{g}^{-1}$ , and iodine value =  $125 \text{ mg-I} \cdot \text{g}^{-1}$ .

### Poultry manure and UCO pre-treatment

The raw poultry manure was mixed with hot water ( $70 \text{ }^\circ\text{C}$ ) at ratio 1:3 (w/w), then screened (1 mm mesh) to remove large solids. Hot water was selected to promote the solubilization of large biopolymers and ensure hygiene (Harris & McCabe 2015; Rodriguez-Verde *et al.* 2018; Spyridonidis *et al.* 2018). The liquid fraction, the so-called DPM, was used in the study. DPM/UCO emulsification was performed at different UCO dosages from 1 to 6% (v/v), using a high-shear emulsifier at 3,000 rpm (IKA-Werke GmbH, Germany). The corresponding mixtures were characterized for emulsion stability (percentage of floating material in graduated cylinders) after five (5) subsequent freeze-thaw cycles.

### Batch anaerobic digestion studies

Mixtures of DPM with different UCO doses were digested in batch anaerobic reactors (2 L working volume) at an initial COD concentration of  $3 \text{ g-COD} \cdot \text{L}^{-1}$ , supplemented with trace elements (Ni, Co, Mo, Fe). The experiments were conducted in triplicate. All batch reactors were equipped with magnetic stirrers, thermal baths with hot water recirculation in double glass jackets, and pH measurement. The pH was maintained at  $7.40 (\pm 0.30)$  and the operating temperature at  $39.0 (\pm 0.7) \text{ }^\circ\text{C}$ . The biogas production from each reactor was measured using an inverse water column, with acidified water to minimize dissolution of  $\text{CO}_2$ . The inoculum was obtained from an anaerobic digester treating animal by-products (Eftaxias *et al.* 2018). Before batch assays, the inoculum was incubated under anaerobic conditions for more than 10 days (without substrate) for full degasification. The ratio of substrate to inoculum (F/M) was kept constant at 0.4 to  $0.5 \text{ g-COD} \cdot \text{g}^{-1}$  MLSS during the study.

### Continuous anaerobic digestion studies

An anaerobic digester (CSTR) with 42 L working volume was used for the study, and maintained at  $38 \pm 1 \text{ }^\circ\text{C}$ , using a thermal bath (LAUDA GmbH, Germany) with hot water recirculation through the reactor double jacket. A paddle mixer was used at 40 rpm. Fresh substrate mixture was prepared daily

and fed into the batch-fed digester once each day. The hydraulic retention time was gradually reduced from 40 to 10 days, corresponding to an OLR increase from 2 to 8 g L<sup>-1</sup> d<sup>-1</sup>. The digester effluent was discharged to a sedimentation tank (4 L working volume), where the anaerobic sludge was separated and recirculated to maintain the MLSS concentration between 15 and 20 g · L<sup>-1</sup>. The digester was inoculated with the same anaerobic sludge used for the batch digestion assays.

### Analytical methods

During digester operation, the quantity of biogas produced was recorded with a wet gas meter (RITTER GmbH, Germany), while the biogas methane and carbon dioxide content were monitored with an infrared biogas analyzer (BINOS – Leybold GmbH, Germany). Samples from the digester influent and effluent, and the mixed liquor, were obtained twice a week for chemical analysis. They were characterized for chemical oxygen demand (COD), TS, VS, pH, electrical conductivity (EC), orthophosphates (PO<sub>4</sub><sup>3-</sup>-P), ammoniacal nitrogen (NH<sub>4</sub><sup>+</sup>-N) and total Kjeldahl nitrogen (TKN), using [APHA Standard Methods \(1998\)](#). The total lipid content was determined using Soxhlet extraction on substrate dry matter. The determination of soluble COD, PO<sub>4</sub><sup>3-</sup>-P, NH<sub>4</sub><sup>+</sup>-N and EC inside the digester was performed after sample centrifugation for 5 minutes at 4,000 rpm. Volatile fatty acid (acetic, propionic, butyric, iso-butyric and valeric acids) concentrations were measured by gas chromatography (Autosystem XL GC, Perkin Elmer Inc, USA) according to the methods described by [Diamantis \*et al.\* \(2006\)](#). The protein content was determined from the organic N content (TKN – NH<sub>4</sub>-N) considering a ratio of 6.25 g-protein · g-organic-N<sup>-1</sup> and a COD value of 1.42 g-COD · g-protein<sup>-1</sup> ([Girault \*et al.\* 2012](#)). The respective coefficient for lipid COD determination was 2.85 g-COD · g · HEM<sup>-1</sup>. The remaining COD was considered to be carbohydrate and lignocellulose species.

## RESULTS

### DPM and UCO emulsion stability

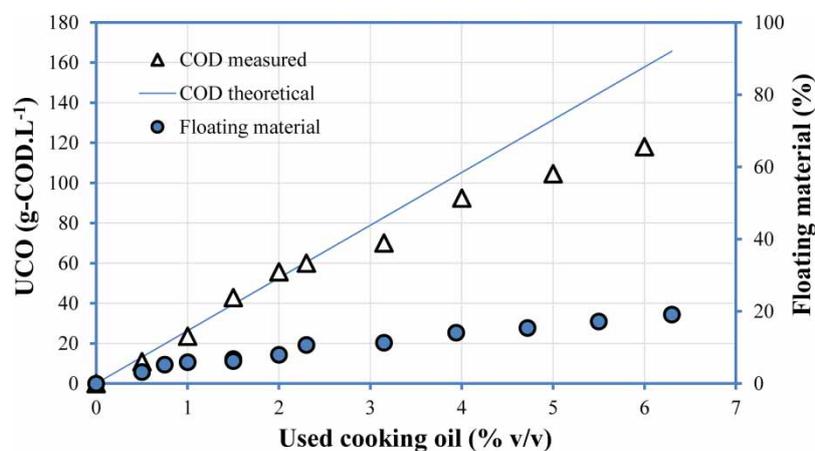
The DPM was characterized by a total COD concentration of 64 g · L<sup>-1</sup>, consisting of 14% lipids and 35% proteins ([Table 1](#)). Emulsification with UCO resulted in an increase of COD by a factor of 1.9 to 2.8, i.e. from 64, initially, to 120 g · L<sup>-1</sup> (at 2% v/v UCO dose), and to 182 g · L<sup>-1</sup> (UCO dose 6%). As the UCO dose was increased, however, the mixture's stability was adversely affected, because the lipids tended to aggregate and float. As shown in [Figure 1](#), the percentage of floating material after five consecutive freeze/thaw cycles increased from 3 to 20% with increasing UCO dosage from 1 to 6% (v/v). Moreover, the emulsion COD remained close to theoretical values when the UCO dose was below 2.3% v/v. As the UCO dose increased from 2.3 to 6.0% (v/v) a significant fraction of the COD (from 10 to 25%) remained as large floating aggregates.

### Batch anaerobic digestion studies

Anaerobic digestion of UCO alone produced biogas at low rates (0.022 L · g-COD<sup>-1</sup> · d<sup>-1</sup>) combined with a 4 to 5 day lag-phase ([Figure 2](#)). Conversely, there was no lag-phase when DPM alone was digested and biogas was produced at a significantly higher rate (0.13 L · g-COD<sup>-1</sup> · d<sup>-1</sup>), similar to the degradation of both acetic and butyric acids – at 0.13 ( $p = 0.94$ ) and 0.11 L · g-COD<sup>-1</sup> · d<sup>-1</sup> ( $p = 0.90$ ), respectively ([Figure 2\(b\)](#)). Indeed, 90% of the biogas produced from DPM alone was recovered within 5 days, compared to 20 days for UCO alone. Both anaerobic digestion tests, however, resulted in similar biogas yield, equal to 0.53 ± 0.01 L · g-COD<sup>-1</sup>.

**Table 1** | Physico-chemical properties of DPM

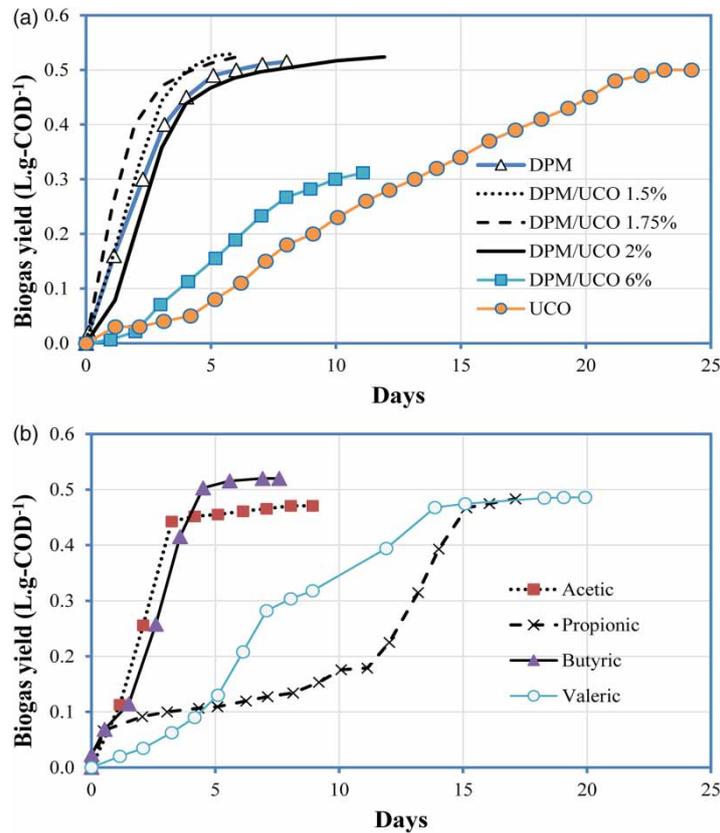
Parameter	Value
pH	6.68 ( $\pm$ 0.78)
EC ( $\text{mS} \cdot \text{cm}^{-1}$ )	22.0 ( $\pm$ 2.1)
COD total ( $\text{g} \cdot \text{L}^{-1}$ )	64.0 ( $\pm$ 8.9)
COD soluble ( $\text{g} \cdot \text{L}^{-1}$ )	31.4 ( $\pm$ 5.2)
TSS ( $\text{g} \cdot \text{L}^{-1}$ )	36.3 ( $\pm$ 16.1)
VSS ( $\text{g} \cdot \text{L}^{-1}$ )	25.4 ( $\pm$ 13.5)
Proteins ( $\text{g} \cdot \text{L}^{-1}$ )	14.2 ( $\pm$ 1.5)
Fat, oil and grease ( $\text{g} \cdot \text{L}^{-1}$ )	3.4 ( $\pm$ 0.6)
$\text{PO}_4\text{-P}$ ( $\text{mg} \cdot \text{L}^{-1}$ )	181 ( $\pm$ 92)
$\text{NH}_4\text{-N}$ ( $\text{mg} \cdot \text{L}^{-1}$ )	1,739 ( $\pm$ 958)
TKN ( $\text{mg} \cdot \text{L}^{-1}$ )	4,640 ( $\pm$ 763)
Na ( $\text{mg} \cdot \text{L}^{-1}$ )	46 ( $\pm$ 9)
K ( $\text{mg} \cdot \text{L}^{-1}$ )	129 ( $\pm$ 30)
Ca ( $\text{mg} \cdot \text{L}^{-1}$ )	33 ( $\pm$ 13)
Mg ( $\text{mg} \cdot \text{L}^{-1}$ )	10 ( $\pm$ 3)
Fe ( $\mu\text{g} \cdot \text{L}^{-1}$ )	760 ( $\pm$ 340)
B ( $\mu\text{g} \cdot \text{L}^{-1}$ )	530 ( $\pm$ 150)
Zn ( $\mu\text{g} \cdot \text{L}^{-1}$ )	403 ( $\pm$ 92)
Mn ( $\mu\text{g} \cdot \text{L}^{-1}$ )	250 ( $\pm$ 180)

**Figure 1** | Effect of UCO dose (% v/v) on the percentage of floating material (%) and the corresponding UCO COD concentration (theoretical and measured).

DPM mixed with UCO doses of 1.5, 1.75 and 2% (v/v) yielded biogas at high production rates, similar to those from DPM alone ( $p = 0.84, 0.88$  and  $0.98$  respectively). This was not the case, however, for the 6% (v/v) mixture, for which a lag-phase of 2 to 3 days was recorded (Figure 2(a)). On the basis of these findings, UCO dosage between 1.5 and 2.0% (v/v) was selected for further study in a continuous (batch-fed) anaerobic digester.

#### Continuous anaerobic co-digestion of DPM and UCO

The CSTR was fed daily with the DPM-UCO mixture (between 1.5 and 2.0% v/v). No digester foaming or sludge flotation was recorded during the study even though the reactor received around 50% of the

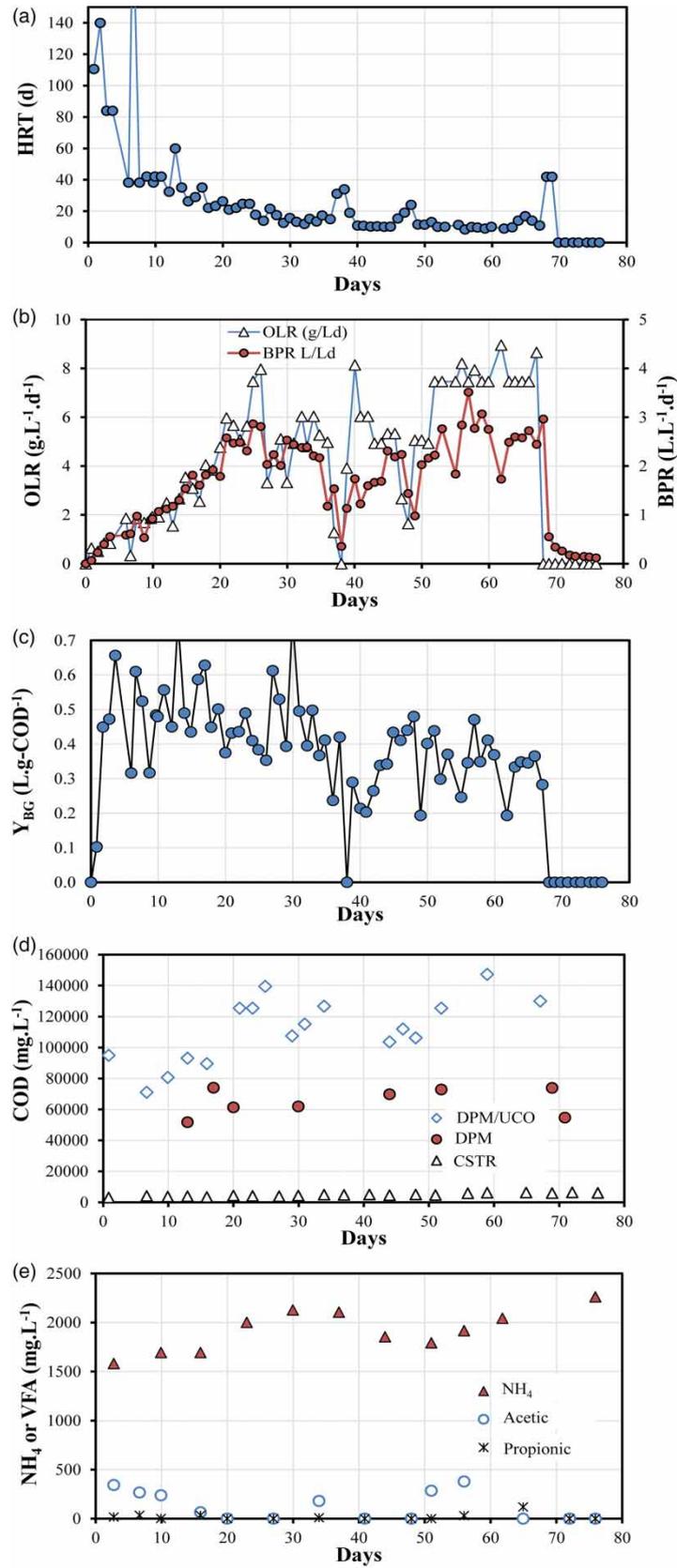


**Figure 2** | Cumulative biogas production during batch anaerobic digestion of (a) UCO, DPM and DPM-UCO mixtures, and (b) acetic, propionic, butyric and valeric acids.

incoming COD as lipids. The biogas production rate increased to  $2.0$  to  $2.5 \text{ L} \cdot \text{L}^{-1} \cdot \text{d}^{-1}$  with increasing OLR, but the biogas yield declined from  $0.45 \pm 0.05$  to  $0.34 \pm 0.07 \text{ L} \cdot \text{g-COD}^{-1}$  when the OLR increased above  $5 \text{ g} \cdot \text{L}^{-1} \cdot \text{d}^{-1}$  (Figure 3). The biogas methane content was  $74.2\%$  ( $\pm 2.9$ ) throughout the study. The supernatant COD (SCOD) was also constant at  $5.3 \pm 0.8 \text{ g} \cdot \text{L}^{-1}$ , throughout the study, corresponding to an SCOD removal efficiency of  $85\%$ . The digester was stable with negligible VFA accumulation ( $<0.5 \text{ g/L}$ ) (Figure 3(d) and 3(e)).

## DISCUSSION

UCO is an energy-rich substrate with high specific methane yield (Labatut *et al.* 2011). Previous studies on anaerobic digestion of UCO alone, however, revealed long digestion periods (25 to 30 days), combined with negligible biogas production (lag-phase) at the start of the batch tests (Labatut *et al.* 2011; Li *et al.* 2011; Fierro *et al.* 2014). This behavior was attributed to long-chain fatty acid (LCFA) accumulation and adsorption onto the anaerobic biomass, causing mass transfer limitations and leading to inhibition (Fierro *et al.* 2014). Similar behavior was observed in this study, during the batch anaerobic digestion of UCO alone (Figure 2(a)). Indeed, upon UCO addition to the batch digesters, individual lipid particles were observed floating on the mixed liquor. Their size decreased gradually with digestion time. Lipids are difficult to biodegrade because of their tendency to form insoluble aggregates that float on water (Eftaxias *et al.* 2018; He *et al.* 2018). He *et al.* (2018) digested UCO as the sole carbon source and reported that the maximum biogas yield –  $1.44 \text{ L} \cdot \text{g} \cdot \text{VS}^{-1}$  (corresponding to  $100\%$  UCO biodegradation), was achieved only during digester operation at the lower OLRs ( $0.5$  to  $1.0 \text{ g} \cdot \text{VS} \cdot \text{L}^{-1} \cdot \text{d}^{-1}$ ), which is indicative of UCO's low biodegradability.



**Figure 3** | Performance of the continuous (batch-fed) anaerobic digester treating the DPM/UCO mixture: (a) hydraulic retention time, (b) organic loading and biogas production rates (OLR and BPR), (c) biogas yield ( $Y_{BG}$ ), (d) influent and effluent COD, and (e) ammonia and VFA concentrations.

DPM, however, was characterized by a large fraction of soluble COD (around 50% of total COD) (Table 1), composed mainly of carbohydrates and proteins, which are readily biodegradable (Bastianelly *et al.* 2010; Metzler-Zebeli *et al.* 2016). Thus, this substrate was beneficial for high-rate biogas production, as shown in both this study's batch anaerobic assays and previous publications (Elasri & Elamin 2016; Rodriguez-Verde *et al.* 2018).

Co-digestion of lipids with poultry manure can decrease inhibitory concentrations of LCFA (by dilution), improve the nutrient balance (e.g. C/N ratio) and attain synergistic effects between microorganisms (Fierro *et al.* 2014; Shah *et al.* 2015; Salama *et al.* 2019). High-shear emulsification was also beneficial in decreasing lipid particle size, promoting solubilization and enhancing substrate availability to microbes (increasing the surface area for enzymatic interaction), because of which high COD removal efficiency and good reaction kinetics and biogas production were achieved. Co-digestion of DPM and UCO in batch assays (at UCO doses between 1.5 and 2.0% v/v), resulted in high biogas production rates with no lag-phase. Increasing the UCO dose further, however (>2% v/v) led to lipid aggregation and flotation (Figure 1), with an extended anaerobic digestion period and a lag-phase, as for UCO alone. This indicates that the rate-limiting step in UCO anaerobic digestion is lipid solubilization.

Fierro *et al.* (2014) showed that batch co-digestion of lipids (from UCO) and pig manure was possible without a lag-phase, compared to lipid waste alone. The authors provide no information on substrate pre-treatment (e.g. mixing or heating), but the proportions of lipid waste and pig manure were variously 1, 4, 7 and 10% (w/w on TS basis), corresponding to 0.14, 0.57, 1.0 and 1.5% (v/v) (i.e. within the optimum UCO dose range of this study). They also reported that continuous (batch-fed) anaerobic digestion of the 1% (v/v) mixture was efficient, without foaming or biomass flotation, as in this study. Ziels *et al.* (2016) co-digested waste restaurant oil with a mixture of primary and secondary sludge, implementing an oil proportion of 13 to 52% (w/w VS basis) (corresponding to 0.4 to 1.4% (v/v), within the optimum range reported here). In this case, VFA concentrations never exceeded 100 mg/L during digester operation, while LCFA remained low (<100 mg · g-TS<sup>-1</sup>).

Wang *et al.* (2013) studied the anaerobic co-digestion of waste activated sludge with grease trap waste. The volumetric proportion of grease waste varied from 10 to 20 and 40% (v/v) (corresponding to 1, 2 and 4% v/v, as the fat, oil and grease (FOG) proportion of the grease waste was 10%). As in this study, stable digester performance was achieved with FOG comprising between 1 and 2% (v/v), while higher FOG doses (4% v/v) resulted in foam build up and decreases in biogas production and methane content. The authors report that the waste activated sludge and grease waste were mixed thoroughly an hour before feeding at 37 °C.

Studies performed with higher proportions of FOG showed a dramatic decline in process efficiency. Li *et al.* (2018) examined batch co-digestion of UCO with food waste at proportions between 33 and 53% (w/w VS basis) (corresponding to 5 to 14% v/v). The food waste and UCO were macerated to between 1 and 2 mm before use. In all of the batch anaerobic digestion tests performed, an initial lag-phase of 15 to 50 days was recorded, with negligible biogas production.

The results of this study show that the mechanisms involved in high-shear emulsification of UCO is lipid disintegration and the formation of small lipid particles, due to the shearing force of the mixer. The small lipid particles are subsequently emulsified and stabilized by the DPM organics (proteins and polysaccharides respectively). Under optimum conditions (i.e. UCO up to 2% v/v) the mixture remains stable, enabling an increase of the surface area available for the bacteria to hydrolyze and metabolize. If the UCO dose is increased further, the mixture's stability is adversely affected; for example, with increased aggregation and flotation. Under optimum conditions, anaerobic co-digestion of UCO and DPM proceeds without inhibition or operating problems.

## CONCLUSIONS

Anaerobic digestion of UCO alone displayed low biodegradability, attributed to low solubility and bioavailability. When the UCO was mixed with DPM, however, up to 2% (v/v), the batch anaerobic digestion process was stable and not inhibited. Further increasing the UCO dose from 2 to 6% (v/v) adversely affected mixture stability and digestibility. Under optimum conditions, the DPM-UCO mixture was digested at OLRs up to  $8 \text{ g} \cdot \text{L}^{-1} \cdot \text{d}^{-1}$ , with negligible VFA accumulation or sludge flotation. DPM is a suitable substrate for UCO co-digestion. This study has shown that, in full-scale anaerobic digestion facilities, by supplementing 2% (v/v) UCO it is possible to double both the organic content and the corresponding biogas yield.

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