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# Biomass adaptation over anaerobic co-digestion of sewage sludge and trapped grease waste

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#### ABSTRACT

The feasibility of sewage sludge co-digestion using intermediate waste generated inside a wastewater treatment plant, i.e. trapped grease waste from the dissolved air flotation unit, has been assessed in a continuous stirred lab reactor operating at 35 °C with a hydraulic retention time of 20 days. Three different periods of co-digestion were carried out as the grease waste dose was increased. When the grease waste addition was 23% of the volatile solids fed (organic loading rate 3.0 kg<sub>COD</sub> m<sup>-3</sup> d<sup>-1</sup>), an increase in methane yield of 138% was reported. Specific activity tests suggested that anaerobic biomass had adapted to the co-substrate. The adapted inoculum showed higher acetoclastic methanogenic and  $\beta$ -oxidation synthrophic acetogenic activities but lower hydrogenotrophic methanogenic activity. The results indicate that a slow increase in the grease waste dose could be a strategy that favours biomass acclimation to fat-rich co-substrate, increases long chain fatty acid degradation and reduces the latter's inhibitory effect.

#### 1. Introduction

Sludge production in the European Union has been increasing for several years. More than 10 million tons dry matter of sewage sludge (SS) were produced in 2006 (Laturnus et al., 2007), representing about 58 kg dry matter per inhabitant-equivalent and year (Mogoarou, 2000). As society demands cleaner water, and because of the strict regulations contained in the 91/271/EEC Council Directive (CEC, 1991), new waste-water treatment plants (WWTPs) will be built and the existing ones will be optimized; this will probably cause a further increase in sludge production.

Sewage sludge (SS) contains a high percentage of organic matter (60–70% of the dry matter) and nutrients such as phosphorous and nitrogen, which can be recycled for agricultural use if the SS is free of heavy metals and other pollutants. The anaerobic digestion process is a well-known technology that improves SS quality for agricultural use, while at the same time producing biogas that can supply between 40–60% of the energy required to run a WWTP (Shizas and Bagley, 2004). Different strategies have been proposed to increase biogas production and optimize SS anaerobic digestion (Appels et al., 2008; Kalogo and Monteith, 2008). One of these is the co-digestion of SS with other organic wastes, while increasing the load of biodegradable organic matter and improving the biochemical conditions of the different microorganism populations

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that develop. Since the optimum carbon-to-nitrogen ratio (C/N) is between 20 and 30 (Parkin and Owen, 1986), and SS has a C/N ratio of between 6 and 16, co-digestion with other organic waste with a high C/N ratio could improve the nutrient balance and increase the amount of degradable carbon and, consequently, the biogas yield (Sosnowski et al., 2007).

Two factors that limit co-digestion are the associated transport cost of co-substrates and the addition of new, external waste to the WWTP. One possible option is to use intermediate waste generated inside the WWTP, such as the grease waste (GW) trapped in the dissolved air flotation (DAF) unit. This would lead to an optimization of the entire plant, since the costs of managing the GW to landfill will decrease, and its high fat content will increase biogas yield.

Various authors have reported increased methane yields during the co-digestion of SS with different types of fats. Davidsson et al. (2008) reported an increase of 9–27% when 10–30% grease, on a volatile solid (VS) basis, was added to an SS anaerobic reactor. Loustarinen et al. (2009) reported an increase of 60% when SS was co-digested with the grease trapped from a meat-processing plant (46% VS added), and Kabouris et al. (2009) found that methane yields were 2.6 times higher when they added oil and grease from restaurants and food outlets (48% total VS load).

Fats are degraded following a specific anaerobic chain reaction and a metabolic route, which is different from that of proteins and carbohydrates. In the first step, the neutral fats are hydrolyzed (lipolyzed) into free long-chain fatty acids (LCFAs) and glycerol,

**Table 1** Characterization of trapped grease waste (GW) and sewage sludge (SS).

| Parameters                       | Units | GW1           | GW2         | GW3           | GW4           | SS             |
|----------------------------------|-------|---------------|-------------|---------------|---------------|----------------|
| TS                               | g/kg  | 146 ± 1       | 160 ± 4     | 126 ± 1       | 75 ± 3        | 32 ± 5         |
| VS                               | g/kg  | 123 ± 1       | 143 ± 3     | 101 ± 1       | 63 ± 2        | $23 \pm 4$     |
| VS                               | %TS   | 84%           | 89%         | 80%           | 84%           | 70%            |
| COD                              | g/kg  | 298 ± 20      | 321 ± 30    | 258 ± 37      | 177 ± 5       | $44 \pm 8$     |
| Fat                              | g/kg  | 47 ± 1        | 100 ± 4     | 38 ± 2        | 15 ± 2        | $0.2 \pm 0.05$ |
| Fat                              | %VS   | 38%           | 70%         | 38%           | 24%           | 1%             |
| SO <sub>4</sub> <sup>2-</sup> -S | mg/kg | 61 ± 4        | 37 ± 1      | 42 ± 3        | 127 ± 1       | 19 ± 0         |
| TKN                              | mg/kg | $4287 \pm 47$ | 3556 ± 51   | $3166 \pm 53$ | $3428 \pm 53$ | $2000 \pm 294$ |
| $NH_4^+ - N$                     | mg/kg | 659 ± 12      | $348 \pm 4$ | 377 ± 8       | 353 ± 24      | 841 ± 109      |
| $NH_4^+$ – $N$                   | %TKN  | 15%           | 10%         | 12%           | 10%           | 42%            |
| C/N                              | g/g   | 20            | 39          | 23            | 10            | 10             |

catalyzed by extracellular lipases. The free LCFAs are converted into acetate and  $H_2$  by acetogenic bacteria through a  $\beta$ -oxidation process, and finally methane is produced by methanogenic bacteria (Masse et al., 2002).

As the fat concentration in SS is generally low, the introduction of fatty wastes into a SS anaerobic reactor can change the biochemical activities of the different groups of anaerobic microorganisms. The acclimation of anaerobic sludge to a specific substrate leads to a new bacterial population that can be different from the mother culture (Gavala and Lyberatos, 2001) or at least result in a new bacterial population distribution (Palatsi et al., 2010).

The aim of this paper is: (i) to characterize and to determine the methane potential of trapped GW from the dissolved air flotation unit of a WWTP, (ii) to assess the feasibility of the co-digestion of SS and GW, and (iii) to analyse biomass adaptation during co-digestion with GW.

#### 2. Methods

#### 2.1. Substrates

In order to characterize the trapped GW from the DAF unit of a WWTP, four samples (GW1, GW2, GW3 and GW4) were taken from four different WWTPs (Barcelona, Spain). The SS used in the anaerobic experiments was a mixture of 70% primary sludge and 30% activated sludge. It was sampled every second week and kept refrigerated at 4 °C. GW2, the co-substrate used in the continuous experiment, was sampled twice (GW2 and GW2') and kept frozen.

The inoculum (In1) used for batch and for the start-up of the continuous reactor was the effluent from a full scale anaerobic mesophilic digester. Biomass adaptation was assessed by comparing the activity of In1 with the adapted inoculum (In2), sampled at the end of the continuous experiment. Inocula were stored at 35 °C before using, in order to avoid the decrease of their activity. Storage time was less than 3 days in all the cases.

#### 2.2. Analytical methods

Total solids (TS), volatile solids (VS), total suspended solids (TSS), volatile suspended solids (VSS), total chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN), ammonia nitrogen (NH<sub>4</sub>\*-N), total and partial alkalinity (TA, PA), sulfate ( $SO_4^{2}$ -S) and fat concentrations were measured according to Standard Methods (APHA, AWWA, WEF, 1995). The elemental composition (carbon (C), nitrogen (N) and hydrogen (H<sub>2</sub>)) was determined by catalytic oxidation combined with gas chromatography (LECO Instruments).

The biogas composition  $(CH_4, H_2, and CO_2)$  and the volatile fatty acids (VFA) (acetate, propionate, iso-butyrate, n-butyrate, iso-valerate and n-valerate) were determined with a gas chromatograph

(CO-300 Varian, USA) using, respectively, a packed column with a thermal conductivity detector, and a capillary column with a flame ionization detector (FID). LCFA concentration was determined in accordance with Palatsi et al. (2009). Samples were lyophilized and digested at 90 °C using chlorotrimethylsilane (CTMS) as a catalyst to form fatty acid methyl ester (FAME), which was then identified and quantified by gas chromatograph (GC 3800 Varian, USA) with a capillary column and a FID detector.

#### 2.3. Anaerobic biodegradability test

The methane potential of the GW and SS were determined by means of anaerobic biodegradability tests based on Campos et al. (2008). Glass vials with a capacity of 1.2 L were filled with 0.5 L of a mixture of inoculum In1 (5 g<sub>VSS</sub>/L), substrate (5 g<sub>COD</sub>/L) and deionized water. The mixture was supplemented with macro/micronutrients (NH<sub>4</sub>Cl, K<sub>2</sub>HPO<sub>4</sub>, MgSO<sub>4</sub>, CaCl<sub>2</sub>, FeCl<sub>2</sub>, H<sub>3</sub>BO<sub>3</sub>, ZnCl<sub>2</sub>, CuCl<sub>2</sub>, MnCl<sub>2</sub>, (NH<sub>4</sub>)6Mo<sub>7</sub>O<sub>2</sub>, CoCl<sub>2</sub>, NiCl<sub>2</sub>, EDTA, HCl, NaSeO<sub>3</sub>, resazurine) and bicarbonate (1 g<sub>NaHCO3</sub>/g<sub>COD</sub>), following (Ferrer et al., 2010). The vials were bubbled with N<sub>2</sub> and placed in an orbital shaker inside a cabin at 35 °C. A control vial without substrate was included to assess the residual methane potential of the inoculum, thereby enabling the net methane potential to be calculated. The methane yield was determined as the final accumulated methane production per initial organic content of the substrate on COD basis (MP<sub>COD</sub>; NL<sub>CH4</sub>/kg<sub>COD</sub>) or VS basis (MP<sub>VS</sub>; NL<sub>CH4</sub>/kg<sub>VS</sub>).

#### 2.4. Continuous experiment

A continuous experiment was carried out in a 7 L continuous stirred tank reactor (CSTR) with a working volume of 5.5 L. The CSTR was operated at mesophilic range (35 °C), with a hydraulic retention time (HRT) of 20 days. The reactor was fed twice a day with a temporized peristaltic pump. Biogas production was measured with a volumetric milligas counter (Ritter Apparatebau GMBH & CO KG, model MGC-10). The influent and effluent characteristics were measured once a week and biogas composition twice a week.

**Table 2**Methane potential (MP) estimated during the biodegradability test and biodegradable COD to VS ratio (COD<sub>AB</sub>/VS) of the substrate tested.

| Waste | NL <sub>CH4</sub> /kg <sub>VS</sub><br>MP <sub>VS</sub> | NL <sub>CH4</sub> /kg <sub>COD</sub><br>MP <sub>COD</sub> | NL <sub>CH4</sub> /kg<br>MP | g/g<br>COD <sub>AB</sub> /VS |
|-------|---|---|-----------------------------|------------------------------|
| GW1   | 483 ± 37  | 215 ± 17  | 69 ± 5                      | 1.5 ± 0.1                    |
| GW2   | 473 ± 53  | 232 ± 17  | 58 ± 7                      | $1.3 \pm 0.2$                |
| GW3   | 529 ± 11  | 207 ± 4   | 53 ± 1                      | $2.0 \pm 0.0$                |
| GW4   | $432 \pm 27$  | 154 ± 10  | 27 ± 2                      | $1.3 \pm 0.1$                |
| SS    | 322 ± 6   | 237 ± 4   | 9 ± 0                       | 1.2 ± 0.0                    |

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