Trace element requirements for the anaerobic digestion of lipid-rich slaughterhouse wastes

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Abstract

In this study lipid-rich slaughterhouse wastes were digested in continuous stirred tanks reactors under a low or a high trace element dosage. The examined wastes were thermal pre-treated and characterized by a COD concentration of 180 ±37 g L^{-1}, consisting 57% of animal fat, 38% proteins. By implementing a high trace element dosage equal to 4 μg Ni, 4 μg Co and 6 μg Mo per g COD influent, it was possible to operate the CSTR digester up to an OLR = 12 g L^{-1} d^{-1}, with a concomitant biogas production rate of 6.5 g L^{-1} d^{-1}. The biogas methane content was 77%, soluble COD inside the reactor remained between 2.5 - 3.5 g L^{-1} while VFA concentrations were negligible (< 0.2 g L^{-1}). When the trace elements dosage was low, the digester showed signs of instability (COD and VFA accumulation, combined with sludge flotation) and whitish particles appeared inside the medium. The biogas yield decreased to 0.20 L g^{-1} COD influent, although the concentrations of individual trace elements inside the digester were between 5 - 20 μg L^{-1}. This is indicative of mass transfer limitations due to lipids adsorption onto the anaerobic biomass.

Concluding, the results of this study are considered important for full-scale anaerobic digesters treating lipid-rich wastes, since process stability and efficiency can be improved with increasing the trace element dosage.

Keywords
fat oil and grease, anaerobic digestion, micronutrients, COD accumulation, sludge flotation.

1. Introduction

Waste lipids are of interest for producing biogas, via anaerobic digestion technologies, their digestion, however, is accompanied with process instabilities such as foaming, accumulation of LCFA, sludge floatation, low biogas yield or even complete digester failure [1; 2]. Poor digester performance is generally attributed to lipids (low) solubility and bioavailability [3], their ability to adsorb onto the anaerobic biomass (disintegrating the flocs and causing sludge flotation) [4], as well as to the bactericidal effects of LCFA on hydrolytic, acidogenic and methanogenic bacteria [5].

Methanogenic archaea play a critical role in lipid digestion and thus optimum conditions for their growth and function should be ensured. Trace elements (such as Ni, Co and Mo) are considered of major importance because they are involved in their enzymes systems, in respiratory processes and cell structural stability [6]. However, mass transfer limitations caused by LCFA adsorption onto the anaerobic biomass can hinder the transport of these compounds across the cell membrane, leading to functional inhibition [7]. Under these conditions, the conversion of acetic acid to methane remains low, which further inhibit the degradation of LCFA. The latter accumulate inside the digester (either as individual precipitates or encapsulating the anaerobic biomass) causing sludge disintegration and flotation and eventually leading to digester failure.

The aim of this study was to investigate the effect of trace elements dosage on the anaerobic digestion of lipid-rich slaughterhouse wastes. For this purpose, CSTR digesters with biogas recirculation were used and process efficiency was evaluated considering COD removal efficiency, biogas production, methane yield, VFA accumulation and sludge flotation.

2. Materials and Methods

2.1 Wastewater origin

Animal wastes (fat tissues, offal, blood) were grinded at < 3 mm and thermally pre-treated at 135 °C for 30 min and 4 bar followed by centrifugation for separating the solid and the liquid fraction. The liquid fraction consisted of emulsified fat and proteins was used for the study (Figure 1).

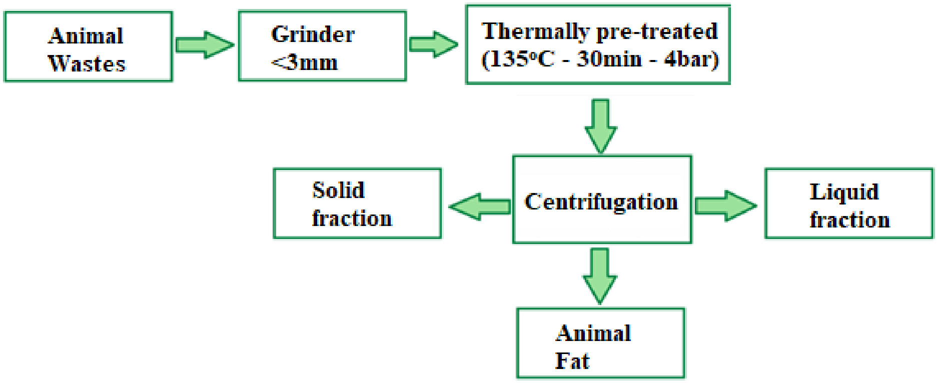

Figure 1. Schematic representation of thermally pre-treated process (Rendering).

2.2 Anaerobic digester design and operation

An anaerobic digester with 42 L working volume was used for the study (Figure 2). The reactor temperature was maintained at 39 (±1) ^{o}C using a thermal bath (LAUDA) with hot water recirculation through the reactor double jacket. Reactor mixing was performed with a paddle mixer operated at low velocity (40 rpm). The influent substrate was stored into completely mixed tank (5L working volume) maintained at room temperature (20 ^{o}C) in order to avoid solidification of animal fat [8]. It was fed into the reactor using a peristaltic pump (Watson Marlow) and a timer. Tap water was added daily at equal amount in order to control the electrical conductivity of the mixed liquor at 16 (±2) mS cm^{-1} and the ammonia nitrogen concentration at 2.2 (±0.3) g L^{-1}. The reactor effluent was connected to a sedimentation tank (4 L working volume) where the anaerobic sludge was separated and recirculated into the system in order to maintain the MLSS concentration equal to 9 ± 2 g L^{-1}. The anaerobic reactors were inoculated with anaerobic biomass obtained from a full-scale facility treating animal wastes.

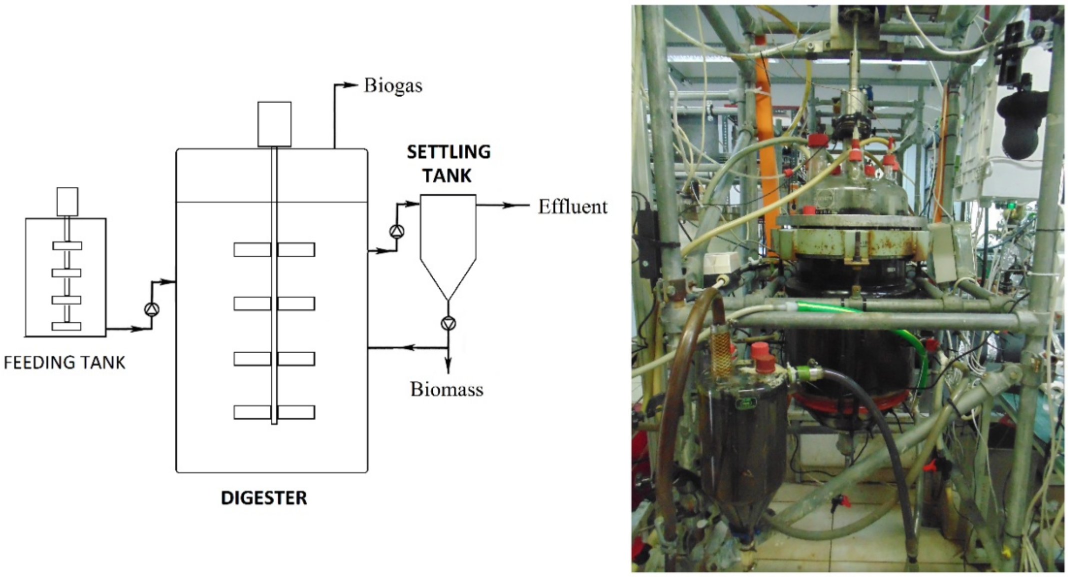

Figure 2. Experimental setup for the continuous anaerobic digestion of thermally pre-treated slaughterhouse wastes.

2.3 Analytical methods

During digester operation, the volume of the wastewater inside the feeding tank was daily recorded to calculate the influent flow rate. The quantity of biogas produced was measured using a wet gas meter (RITTER). The biogas methane and carbon dioxide content were monitored with an infrared biogas analyzer (BINOS). Samples were obtained twice a week from the digester influent, effluent and the mixed liquor for chemical analysis. Digester influent and effluent samples were characterized for Chemical Oxygen Demand (COD), total and volatile solids (TS, VS), pH, electrical conductivity (EC), phosphates (PO43--P), ammonia (NH4+-N) and Total Keldhal Nitrogen (TKN), according to the Standard Methods [9]. The measurements of pH and electrical conductivity were performed using a pH- (Metrohm 632) and EC-meter (CM 35, Crison), respectively. The total lipid content was determined through Soxhlet extraction on substrate dry matter. The substrate was dried at 105 ^{o}C and the extraction was carried out by using hexane/MTBE (80/20) for 4.5 h. After evaporation of the solvent, the hexane extractable material (HEM) was determined by gravimetry. The determination of soluble COD, PO_4^{3-}–P, NH_4^+–N and EC inside the digester was performed after sample centrifugation for 5 min at 4000 rpm. Volatile Fatty Acids (VFA) (acetic, propionic, butyric, iso-butyric and valeric acid) concentrations were measured through gas chromatography (Perkin Elmer Auto system XL), according to [10]. The trace element concentrations were determined in filtered samples (pore size 0.20 μm) by using ICPMS (Thermo ICAP – QC ICP – MS). The wastewater protein content was determined from the organic N content (TKN subtracted by NH4-N) considering a ratio of 6.25 g of protein per g of organic N and a COD value of 1.42 g COD per g of protein [11]. The respective coefficient for lipid COD determination was 2.65 g COD per g of HEM. The remaining COD was considered to be carbohydrates.

3. Results and Discussion

3.1 Waste characteristics

The thermally pre-treated slaughterhouse wastes consisted of a yellowish/ whitish-brown viscous liquid that solidified in the refrigerator (4 ^{o}C) but remained liquid at room temperature and was efficiently homogenized using the stirrer apparatus. When mixing was out of operation, the emulsified animal fat coagulated slowly, and flocs tended to float, but this was reversible upon initiation of mixing. They were characterized by a COD concentration of 180 ± 37 g/L, consisting 57% of animal fat, 38% proteins and the remaining carbohydrates, originating from the animal digestive track content (Table 1).

Table 1. Physicochemical properties of thermally pre-treated slaughterhouse wastes.

Parameter	Value
COD total (g/L)	180 (±37)
TS (g/L)	96 (±24)
pH (-)	6.19 (±0.27)
EC (mS/cm)	13.9 (±2.9)
COD proteins (g/L)	70 (±9)
COD lipids (g/L)	110 (±27)
COD carbohydrates (g/L)	<10
Ni (μg L^{-1})	8-28
Co (μg L^{-1})	3-16
Mo (μg L^{-1})	1.5-8.5
Fe (μg L^{-1})	200-3300

3.2 Digester performance under a low trace element dosage

Firstly, the digester was operated under a low trace element dosage (days 0-100). It was started-up at OLR = 2.0 (±0.2) g L^{-1} d^{-1}, however, biogas production remained low (Figure 3a) and the concentrations of soluble COD increased rapidly from 1.3 to 5.8 g L^{-1} (Figure 3b). This was combined with severe sludge floatation (see Figure 4) and therefore the OLR was immediately decreased to 1.0 (±0.3) g L^{-1} d^{-1} which stabilized digester performance. Foaming was eliminated, and the concentrations of soluble COD returned to 2.4 (±0.1) g L^{-1}. However, with gradually increasing the OLR up to 6 g L^{-1} d^{-1} (day 0 to 60) the process was accompanied with COD accumulation, followed by extreme sludge flotation events (e.g. on days 40 and 59 and headspace of the reactor was full of foam). Moreover, at the higher OLR tested small whitish particles (< 1 mm) appeared inside the mixed liquor and sludge settling properties deteriorated.

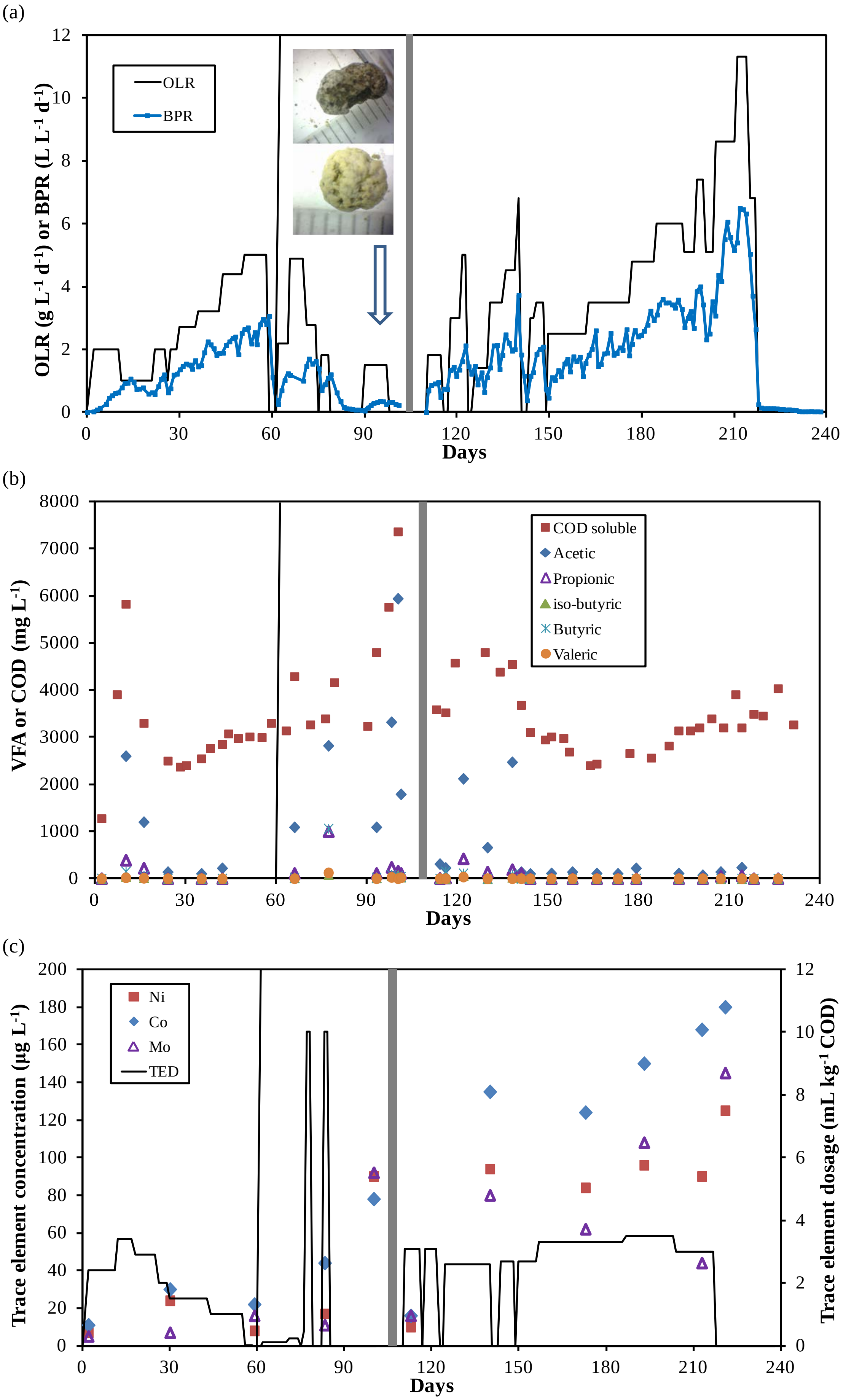

Figure 3. Variation of the (a) organic loading rate (OLR) and biogas production rate (BPR), (b) VFA and COD soluble concentrations and, (c) trace element dosage and the concentrations of Ni, Co and Mo inside the digester during the study.

From day 60 the supplementation of Ni, Co and Mo was interrupted and consequently there was a dramatic decline in process efficiency. Whitish particles accumulated again (see Figure 4), this time increasing both in size and number while the concentration of acetic acid increased immediately after start-up. Considering the above, the digester was set in operation at low OLR = 1.5 (±0.1) g L^{-1} d^{-1}, however, soluble COD and acetic acid accumulated again, and the anaerobic sludge became grey from a previous black appearance. The biogas production rate decreased to less than 0.3 L L^{-1} d^{-1}, corresponding to a biogas yield of 0.20 L g^{-1} COD influent. Large solid particles (0.5-1.0 cm size) were evidenced floating on top of the digester liquor (see Figure 4). Based on these findings, it was decided that the process was inhibited, and further reactor operation was not possible.

Figure 4. Photographic representation of whitish particles and foaming events during the anaerobic digestion of thermal pre-treated slaughterhouse wastes under a low trace element dosage.

3.3 <u>Digester performance under a high trace element dosage</u>

During the second experimental period (days 110 - 230), the start-up phase displayed similar performance, with COD accumulation and VFA increase, comparable to the previous. However, after 1 HRT of wastewater volume treated (from day 138 and on), the concentrations of soluble COD started to decrease from 4.8 to 3.0 g L^{-1} and the VFA concentrations remained low (<300 mg COD L^{-1}) (Figure 3b). From day 160 (2 HRT wastewater volumes treated) foaming was eliminated and the digester displayed an outstanding performance, with negligible COD accumulation, despite that the OLR was gradually increased to 6-8 g L^{-1} d^{-1} (Figure 3a). The biogas production showed a linear correlation (R^2 = 0.96) with the applied OLR indicating that the daily influent COD load was completely converted to biogas. In this period, the biogas yield coefficient was 0.53 L g^{-1} COD influent and the COD removal efficiency remained higher than 96%. The biogas methane content was not affected by the gradually increasing OLR and remained at 77 ±3%.

4. Conclusions

- Trace elements play a critical role in anaerobic digestion since they affect the function and activity of methanogenic archaea.
- Implementing a high trace element dosage proportional to the applied OLR (4 μg Co, 4 μg Ni and 6 μg Mo per g COD feed) resulted in stable digester performance at high OLR (up to 10-12 g L^{-1} d^{-1}).
- COD accumulation remained low, VFA concentrations were negligible and high biogas production (up to 6.5 L^{-1} L^{-1} d) and methane content (77%) were achieved.
- Under a low trace element dosage, the anaerobic digestion process encountered major instabilities as evidenced by severe sludge flotation, accumulation of COD and VFA and the formation of white precipitates inside the mixed liquor.

Acknowledgements

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