Anaerobic digestion of long-chain fatty acids (oleic, palmitic, stearic) with whey protein as the emulsifier

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Abstract
Long chain fatty acids are major lipid constituents. In this study, anaerobic digestion of oleic, palmitic or stearic acid (5 g/L each) emulsified with whey protein (20 g/L) was examined in continuous (batch-fed) stirred tank digesters with 2 L working volume. Anaerobic co-digestion of oleic acid displayed high biogas yield (0.47 L/gCOD), compared to palmitic (0.42 L/gCOD) and stearic acid (0.30 L/gCOD). Oleic acid, despite its high biodegradability resulted in major inhibition of the acetoclastic methanogens, as demonstrated by VFA accumulation and by the methanogenic activity assay. Biogas production from palmitic acid was stable, with a biogas yield close (90%) to the theoretically expected values. This was not the case however for stearic which displayed negligible biodegradability. Application of the ADM1 revealed the maximum degradation rate constant of each LCFA. Based on the results of this study it can be concluded that stearic acid degradation is the rate limiting step of the anaerobic digestion process, and this attributed to its low solubility and thus bioavailability.
Keywords: anaerobic digestion; biogas; LCFA; fat oil and grease; ADM1.

1. Introduction

Long chain fatty acids (LCFA) are major lipid constituents. Their anaerobic degradability, however, is accompanied with process instabilities, such as foaming, accumulation of LCFA, sludge floatation, low biogas yield or even complete digester failure (Lalman and Bagley, 2001). Poor digester performance is generally attributed to the low solubility and bioavailability of LCFA, their ability to adsorb onto the anaerobic biomass, disintegrating the flocs and causing sludge flotation, as well as to the bactericidal effects on hydrolytic, acidogenic and methanogenic microorganisms (Pereira et al, 2005).

In this study we examined the anaerobic digestion of oleic, palmitic and stearic acids in batch and continuous (batch-fed) stirred anaerobic reactors. The LCFA were initially emulsified with whey in order to promote disintegration and solubilization of the lipidic substrate. Process efficiency was evaluated according to biogas production rate, methane yield, effluent quality, VFA accumulation and degree of foaming. The results from the lab-scale digesters were further modelled using ADM1 in order to quantify the kinetics of individual LCFA degradation.

2. Materials and Methods

2.1. Substrates and inoculum

Oleic, palmitic and stearic acid were supplied from Sigma Aldrich. Palmitic and stearic acids were solid at room temperature and their melting point was between 62-67 and 65-72 ^{o}C respectively. Whey protein was supplied from Pulsin**.** The anaerobic inoculum used for the study was obtained from a full-scale facility treating animal by-products (Eftaxias et al., 2018).

2.2. Emulsification pre-treatment

Emulsification was performed using a high-shear emulsifier (IKA T-25 model) at 6000 rpm for 5 min while the mixture was pre-heated at 75 ^{o}C to optimize the procedure. The prepared emulsions consisted of 5 g of each LCFA and 20 g protein. The corresponding mixtures were characterized for emulsion stability (percentage of floating material in graduated cylinders) after five (5) subsequent freeze-thaw cycles.

2.3. Batch anaerobic digestion studies

Raw and emulsified LCFA were digested in batch anaerobic reactors (2 L working volume) at an initial COD concentration of 3 g/L. The experiments were conducted in triplicate. All batch reactors were equipped with magnetic stirrer, thermal bath with hot water recirculation in double glass jacket and pH measurement. The biogas production from each batch reactor was measured using an inverse water column, with acidified water to avoid dissolution of the biogas CO_2.

2.4. Continuous anaerobic digestion studies

Three anaerobic reactors were used for the study with a working volume of 2 L. The reactors were operated in parallel with different LCFA. The digesters temperature was maintained at 39 ^{o}C, using a thermal bath (LAUDA) with hot water recirculation through the reactor double jacket. Mixing was performed with a magnetic stirrer at 200 rpm. The substrate mixture was prepared daily and it was fed into the digester in fed-batch mode (once per day) but occasionally, mixed liquor was removed and sludge was recirculated to keep the digesters' volume and MLSS concentration varying between 1.5-2.5 L and 8-12 g/L respectively. The organic loading rate was gradually increased from 2 to 6 g/Ld. Macro- and micro-

nutrients were supplemented according to Eftaxias et al. (2018).

2.5. ADM1 application

The IWA ADM1 (Batsone et al., 2002) was used to simulate the response of the continuously run bioreactors to the varying operational conditions (increasing loading, varying volume and sludge recirculation). Whey and LCFA were introduced as protein and fatty acids respectively. The nitrogen content was calculated based on the amino acid content of the whey protein. All kinetic parameters were kept constant, except from the maximum specific rate constant of the fatty acid degradation rate, which were adjusted to fit the biogas production rate. The software Aquasim 2.0 was used to perform all simulations.

3. Results and Discussion

During batch anaerobic digestion (Figure 1), the higher biogas production rate was recorded for oleic acid, followed by palmitic and stearic acid. Indeed, biogas production from stearic acid was such slow, indicating that the rate limiting step in the anaerobic digestion process is stearic solubilization (Lalman and Bagley, 2001).

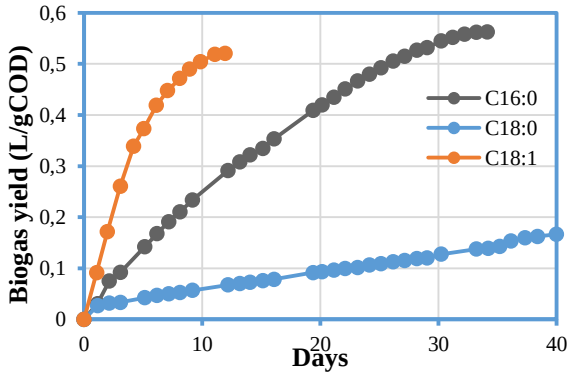

Figure 1. Cumulative biogas production during batch anaerobic digestion of oleic, palmitic and stearic acids as the sole carbon source.

3.2. Continuous anaerobic digestion of LCFA and whey

Continuous (batch-fed) anaerobic digestion (Figure 2) of emulsified oleic acid displayed high biogas yield (0.47 L/gCOD), compared to palmitic (0.42 L/gCOD) and stearic acid (0.30 L/gCOD). Biogas production from palmitic acid was stable, with a biogas yield close (90%) to the theoretically expected values. COD and VFA accumulation inside the digester remained negligible, however, severe foaming was recorded during continuous digester operation. Biogas yield from emulsified stearic acid remained low (25-30% of the theoretically expected values), with negligible COD and VFA accumulation (Figure 2).

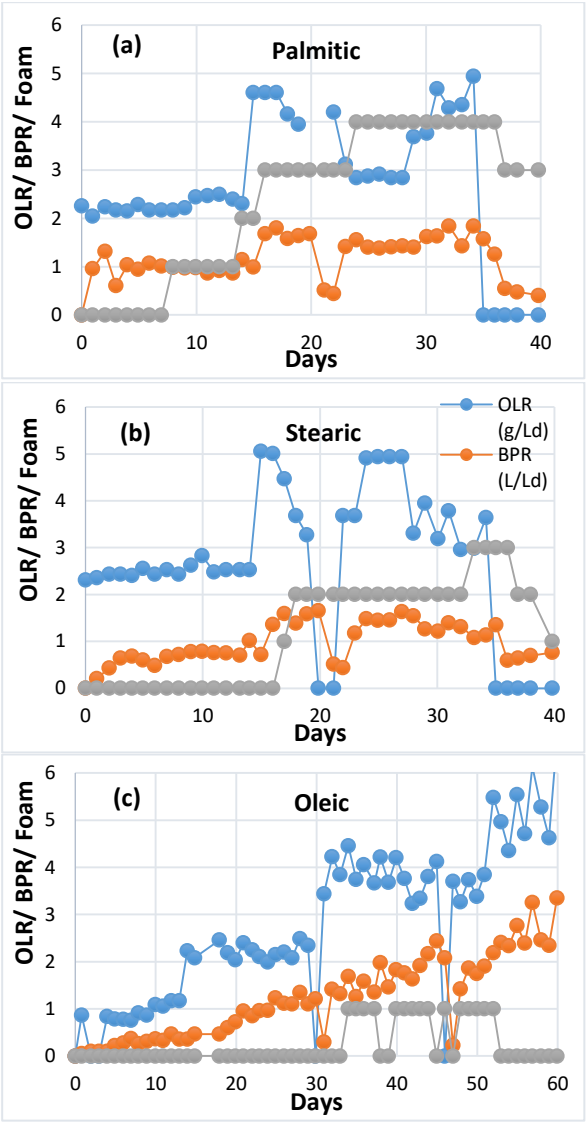

Figure 2. Organic loading rate (OLR in g/Ld), biogas production rate (BPR in L/Ld) and foaming degree (in cm), during continuous (batch-fed) anaerobic digestion of emulsified (a) palmitic, (b) stearic and (c) oleic acid.

The CSTR received daily the DPM-UCO mixture (between 1.5-2.0% v/v). No digester foaming or sludge floatation was recorded during the study despite that the anaerobic reactor received around 50% of the incoming COD as lipids. The biogas production rate displayed an increase up to 2.0-2.5 L L^{-1} d^{-1} with increasing the OLR, however, the biogas yield declined from 0.45±0.05 to 0.34±0.07 L g^{-1} COD when the OLR increased > 5 g L^{-1} d^{-1} (Figure 3). The biogas methane content remained equal to at 74.2 (±2.9) %. Supernatant COD (SCOD) was also constant at 5.3±0.8 g/L, throughout the study, corresponding to a SCOD removal efficiency of 85%. The digester was stable with negligible VFA accumulation (<0.5 g/L).

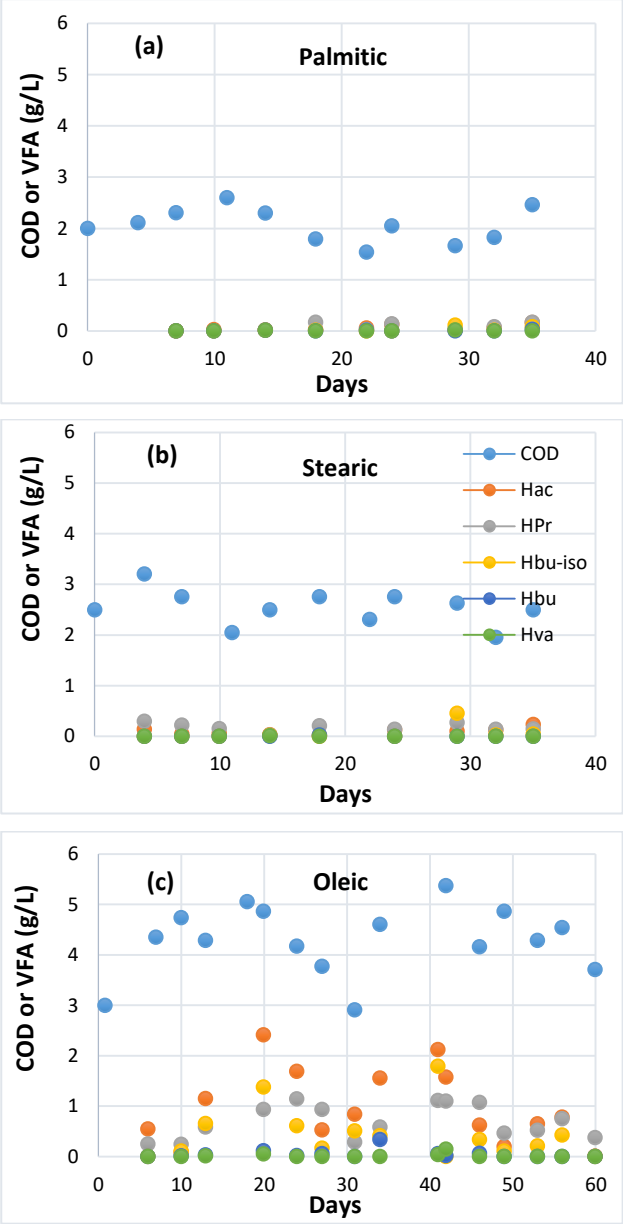

Figure 3. Digester supernatant COD and VFA concentrations, during continuous (batch-fed) anaerobic digestion of emulsified (a) palmitic, (b) stearic and (c) oleic acid.

Table 1. Maximum uptake rate for LCFA degrading microorganisms (km_fa) based on ADM1 modelling.

Parameter	Value
Oleic (kg/kg d)	6.0
Palmitic (kg/kg d)	2.0
Stearic (kg/kg d)	1.5

4. Conclusions

Based on the results of this study it is can be concluded that LCFA solubility (and thus bioavailability) play a critical role in the anaerobic digestion process. Emulsification pre-treatment can increase the rate of biogas production. Stearic acid display low biogas production and this is attributed to low solubility and low emulsion stability.

Acknowledgements

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