ALLEVIATION OF UNSATURATED FATTY ACID INHIBITION DURING ANAEROBIC DIGESTION OF TALLOW

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Background

Anaerobic digestion of tallow wastes has great potential for usable energy production. The process can be economically favourable but system complexities warrant close control at each stage to achieve maximum methane production. The process has three main stages:

1. Fermentative bacteria enact triglyceride hydrolysis to produce long chain fatty acids (LCFA) + glycerol.
2. Syntrophic acetogenic bacteria produce VFA'S + CO_2 + formate/H_2 via beta-oxidation of LCFA'S, eventually to acetic acid.
3. Methanogenic bacteria covert acetic acid to CH_4 + CO_2 and also CO_2 + H_2 into CH_4 + H_2O.

All lipid components and bacterial populations present or formed within the system must be in balance with each other throughout the process before successful methane generation is achieved. Hydrolysed lipid break-down products may be toxic to the degradative bacteria and inhibit their actions on the lipid. LCFA have been implicated in the inhibition of several groups of bacteria during lipid degradation, particularly the syntrophic and methanogenic populations This leads to substantial lag phases (often weeks or months) before gas production starts. The mechanism(s) postulated for alleviation of the temporary inhibitionn of the various bacterial groups are not universal. Oleic acid can be degraded to stearic and palmitic although recently palmitic and myristic were reported as products in unacclimated reactors fed oleate. No LCFA degradation products were observed when these cultures were presented with stearate, though the stearate was slowly removed. Yet In other studies stearate was observed to degrade at the same rate as oleate.

Objectives

To determine levels of main lipid breakdown components: mono, di, triglycerides and free fatty acids (LCFA) in a anaerobic digester using sludge inocula unadapted to tallow addition prior to gas production and to investigate the alleviation of the inhibitory effects of stearic, oleic and linoleic acids towards butyrate fermenting bacteria in tallow acclimated sludges.

Methods

Samples were analysed from unadapted anaerobic digesters fed with 20g/L tallow emulsified with 8% palmitate and from addition of 30mMol/L stearate, oleate or linoleate to digesters (adapted to tallow) to assess their influence on butyrate fermentation.

VFA determinations: Acetate and butyrate levels were monitored by gas chromatography (Broughton et al. (1998)).

TLC profiles: Digester lipid components were screened by thin-layer chromatography on silica gel plates using hexane: diethyl ether: acetic acid (74:25:1 and 24:75: 1) with 2,7-dichlorofluoroscein or phosphomolybdate as indicator.

LCFA levels: Concentrations of free and esterified fatty acids were assessed by gas chromatography (Birch et al. (1998)).

Confirmation: Fatty acids were identified by gas chromatography-mass spectrometry of their TMS derivatives (Hudson et al. (1995)).

Results and discussion

For reactors unadapted for tallow (Table 1) an inhibition of methane generating bacteria occured (lag phase). This can last up to 60 Days.

- Mono and diglycerides did not persist past Day one.
- Triglyceride lipid levels declined rapidly from Day zero and remained at background levels showing that hydrolysis occurs rapidly after the initial addition of tallow.
- LCFA levels increased rapidly and remained at levels similar to total lipid addition values until gas production commenced.
- Levels of oleic acid declined rapidly relative to palmitic and stearic acids, which increased up to gas production. Unsaturated LCFA levels fell to less than 10% of initial total LCFA levels prior to gas production (figure 1).
- Thus high concentrations of LCFA upset the digestion process and reactors required microbially mediated detoxification of LCFA for recovery, when 2 x Oleate ----> Stearate + Palmitate ; (see Broughton et al. 1998)

Acetogenic + formate/H_2 utilising methanogenic bacteria are inhibited by free LCFA.

- Relative sensitivity is: C 18:2 > C 18:1 > C 18:0 (Table 2).
- During the butyrate fermentations, stearate remained at the pre-butyrate addition level while both oleate and linoleate underwent metabolism. Oleate was converted to hydroxystearate over the course of acetate formation (200 hr; figure 2) while linoleate appeared to produce oleate , which degraded without hydroxy formation. Palmitate and ketostearate were possible products. Little butyrate fermentation was recorded in the presence of 30mMol/L linoleate however when the amount of linoleate was reduced to 10mMol/L the fermentation was complete over the time course of the experiments (200 hr).

Pertinent literature

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Table 1 Free LCFA (g/L), VFA (g/L) and Methane levels (g/Day) in the Unadapted Reactor

DAY	0	5	10	15	20	25	30	35	40
LCFA	3.7	9.0	11.8	12.0	12.1	12.0	12.0	11.8	8.5
VFA	1.8	3.0	6.0	6.0	7.0	7.1	7.1	6.2	5.8
Methane	0	0	0	0	0	0	0	0	0.1
DAY	45	50	55	60	65	70	75	80	85
LCFA	3.8	0.5	0.3	0.1	0.1	0.1	0	0	0
VFA	7.0	8.2	4.3	2.1	1.0	0.5	0.3	0.1	0
Methane	0.1	1.8	4.5	3.0	2.2	1.8	1.5	1.0	0.3

Table 2 Cumulative Acetate (mMol/L) Production from Butyrate (30 mMol/L) Fermentation in the presence of added LCFA (30mMol/L) in Tallow Adapted Reactors

Time (H)	20	40	60	80	100	120	140	160	180	200
Control	18	38	60							
Stearate	12	22	30	40	50	60				
Oleate	2	3	5	10	18	27	35	45	54	60
Linoleate	2	2	3	5	7	9	11	14	17	20

Figure 1 LCFA Fractional Composition - Unadapted Reactor

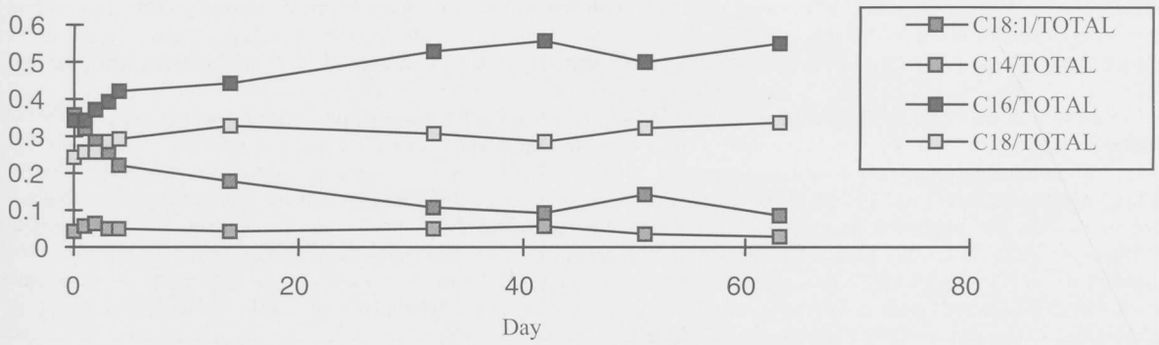

Figure 2 Oleate Trial - adapted Reactor