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ANAEROBIC DEGRADATION AND METHANOGENIC INHIBITORY EFFECTS OF OLEIC AND STEARIC ACIDS

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Abstract—Oleic acid, an 18 carbon acid with one double bond (C18 : 1) was degraded anaerobically to palmitic (C16 : 0) and myristic (C14 : 0) acid by-products at 21°C by a culture unacclimated to long-chain fatty acids. These by-products were degraded to acetate and ultimately to methane. In comparison, no long-chain fatty acid by-products were observed in unacclimated anaerobic cultures receiving stearic (C18 : 0) acid although slow removal of stearic acid occurred. Oleic acid concentrations above 30 mg l^{-1} inhibited acetate degradation but stearic acid up to 100 mg l^{-1} did not inhibit aceticlastic methanogenesis. Hydrogenotrophic methanogenesis was slightly inhibited by oleic and stearic acids. A thermodynamic basis for comparing anaerobic C18 acid degradation and predicting by-products is presented.

Key words—anaerobic, oleic (C18 : 1) acid, stearic (C18 : 0) acid, biodegradation, inhibition

INTRODUCTION

Oleic acid (an 18 carbon acid with one double bond, designated C18 : 1) is primarily found in olive, pecan and teaseed oils (Sonntag, 1979) while stearic acid (C18 : 0) is present in cocoa and tallow (O'Brien, 1998). Under anaerobic conditions these long-chain fatty acids (LCFAs) are produced when fats and oils are hydrolyzed. LCFAs can be slowly degraded under anaerobic conditions to shorter chain acids, but may also inhibit anaerobic microbial activity (Koster and Cramer, 1987; Hanaki *et al*., 1981). The slow degradation of LCFAs and the potential inhibition of other microbial activity by these compounds must be considered when designing and operating anaerobic treatment systems for wastewaters containing vegetable oils.

Oleic and stearic acids are highly insoluble in water, with aqueous solubilities of only about 3 mg l^{-1} at 20°C (Ralston and Hoerr, 1942). Nevertheless, many previous investigations using acclimated cultures considered much higher concentrations: 1800 mg l^{-1} oleic acid incubated at 37°C (Novak and Carlson, 1970); 1000 and 3000 mg l^{-1} oleic acid incubated at 35°C (Canovas-Diaz *et al*., 1991); and 370 mg l^{-1} stearic acid incubated at 55°C (Angelidaki and Ahring, 1995). Degradation products were observed from oleic acid, primarily C16 and C14 acids but no LCFA products were detected from stearic acid (Novak and Carlson, 1970; Angelidaki and Ahring, 1995). Such high concentrations of LCFAs were likely present in emulsion or adsorbed to solids. Lower concentrations much nearer to the solubility limit that may provide a better representation of the underlying degradation process have not been investigated.

Although degradable, LCFAs can inhibit the activity of anaerobic microorganisms, in particular aceticlastic methanogenic organisms. For example, oleic acid at concentrations from 300 to 1500 mg l^{-1} significantly inhibited aceticlastic methanogens at 30°C (Koster and Cramer, 1987) but the effect on hydrogenotrophic methanogens was not examined. At 55°C, 100–1000 mg l^{-1} oleic acid inhibited acetic acid removal as did greater than 300 mg l^{-1} stearic acid (Angelidaki and Ahring, 1992). The impact on hydrogenotrophic methanogenesis was not determined in these studies, even though one-quarter to one-third of methane produced in an anaerobic system is produced by those organisms (Hickey *et al*., 1987).

Some evidence that LCFAs may inhibit hydrogenotrophic methanogens has been reported. A mixture of LCFAs inhibited hydrogenotrophic methanogens at 37°C, but the inhibition was less than that observed for aceticlastic methanogens (Hanaki *et al*., 1981). Additionally, linolenic (C18 : 3) acid was also observed to inhibit hydrogenotrophic methanogens at 39°C (Demeyer and Hendrickx, 1967). However, the inhibitory properties of oleic or stearic acids on hydrogentrophic metha-

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nogenesis have not been examined, even though the inhibition of hydrogen consumption may indirectly impact the degradation of LCFAs.

Because of processing requirements and geographical location, effluents containing fats and oils from food processing facilities will have variable temperature ranges. For example, in comparison to effluents from slaughter-houses in Australia which are between 30 and 35°C, those in Europe are at approximately 20°C (Johns, 1995). The responses of anaerobic systems to LCFAs at temperatures lower than 30°C have not been well documented, although the degradation of mixed LCFAs was observed to cease at 15°C during anaerobic sludge digestion (O'Rourke, 1968). The effects of LCFAs on anaerobic organisms are not known under temperature conditions of approximately 20°C and the impact of oleic and stearic acids on aceticlastic and hydrogenotrophic methanogens at lower temperatures has not been reported.

The first objective of this work was to examine the degradation of lower concentrations ($\leq$100 mg l^{-1}) of oleic and stearic acids at 21°C in cultures acclimated to glucose. Degradation byproducts were also determined. The second objective was to investigate inhibitory effects of lower concentrations ($\leq$100 mg l^{-1}) of oleic and stearic acids on both aceticlastic and hydrogenotrophic methanogenesis at 21°C in cultures acclimated to glucose. The final objective was to develop a thermodynamic basis for comparing the anaerobic degradation of differently saturated C18 acids.

EXPERIMENTAL METHODS

Inoculum source

Experiments were conducted using a 1:6 mixture of digester sludge and granulated anaerobic biomass from the Toronto Main Treatment Plant and a food processing plant in Cornwall, ONT as described previously (Lalman and Bagley, 2000). The mixture contained approximately 20,000 mg l^{-1} volatile suspended solids (VSS) and was maintained with 1000 mg l^{-1} glucose (BDH, Canada) in a 4-l semi-continuous reactor (designated Reactor A) at 21°C. Inoculum from Reactor A was diluted with basal medium (Lalman and Bagley, 2000) into a second 4-l semi-continuous reactor (Reactor B) to achieve 1500 mg l^{-1} VSS. Reactor B was maintained with 1000 mg l^{-1} glucose every 5 to 6 days (time when acetate and gas production measurements indicated that all glucose and byproducts were consumed) and served as the biomass source for all measurements.

Oleic and stearic acid studies

Oleic and stearic acid degradation and inhibition studies were conducted in an identical manner to previous linoleic acid degradation and inhibition studies (Lalman and Bagley, 2000). Serum bottles (160 ml) were prepared under a 70% N_2/30% CO_2 atmosphere and received 100 ml of liquid which included either 96, 98 or 100 ml of culture from Reactor B, depending on the condition examined. The bottles were capped with Teflon®-lined silicone rubber septa and aluminum caps and covered in aluminum foil to inhibit photosynthetic activity and photodegradation. Agitation in an orbital shaker (Lab Line Instruments) at 200 rpm and 21°C $\pm$ 1°C was performed for the duration of each study. To avoid the formation of a negative pressure in the headspace during sampling, culture bottles received 20 ml overpressure of 70% N_2/30% CO_2 immediately after inoculation. Liquid and headspace samples were periodically withdrawn to measure selected parameters. At the completion of each experiment, culture bottles were sacrificed to measure pH, alkalinity (as $CaCO_3$), total suspended solids (TSS) and VSS.

Because the aqueous solubilities of oleic and stearic acids are approximately 3 mg l^{-1} (Ralston and Hoerr, 1942), pure oleic and stearic acids do not disperse well in serum bottles. Therefore, for this work, diethyl ether (Aldrich Canada) stock solutions of each acid at concentrations of 5000 mg l^{-1} were prepared. The use of diethyl ether to facilitate precise addition of LCFAs and adequate dispersion in serum bottles was described previously (Lalman and Bagley, 2000).

Volumes of LCFA stock solution upto 2 ml were added to culture bottles to provide initial LCFA concentrations of 0, 10, 30, 50 and 100 mg l^{-1} oleic acid or stearic acid. Bottles receiving less than 2 ml of LCFA stock solution received additional diethyl ether so that the total volume of LCFA stock solution and diethyl ether added was always 2 ml and the concentration of diethyl ether in any culture was always 14.2 g l^{-1}. There was no evidence of diethyl ether degradation during the experiments.

For the inhibition studies, either acetic acid or hydrogen was added to culture bottles in addition to the LCFA. Sufficient (2 ml) acetic acid stock solution (5000 mg l^{-1} in deionized water) was added to provide an initial acetic acid concentration of 100 mg l^{-1}. Sufficient hydrogen (6 ml of gas) was added to the headspace to provide a partial pressure of 10.13 kPa.

After adding the diethyl ether and acetic acid stock solutions, additional $FeCl_2$ and Na_2S were added to ensure anaerobic conditions in the culture bottles throughout the course of the experiment (indicated by the resazurin dye remaining colorless). All conditions receiving LCFA were conducted in triplicate. Controls (with and without diethyl ether, acetic acid and/or hydrogen) receiving no LCFA were conducted in duplicate.

Analytical methods

Periodically, 2-ml mixed liquor samples were withdrawn from the serum bottles and split into two 1-ml aliquots, one for the measurement of volatile fatty acids (VFAs) and the other for the measurement of LCFAs. The VFA aliquots were diluted with 2-ml deionized water, centrifuged at 1750 g for 5 min and the centrate analyzed using ion chromatography with a conductivity detector as previously described (Lalman and Bagley, 2000). The effective detection limits (incorporating dilution) were 0.2 mg l^{-1} for propionic, *i*-butyric, *n*-valeric and *i*-valeric acid; 0.3 mg l^{-1} for acetic and *n*-butyric and 0.4 mg l^{-1} for hexanoic acid.

The LCFA aliquots were placed into a 5 ml-vial, extracted with 2-ml of a 50:50 hexane:methyl tertiary butyl ether (MTBE) mixture and analyzed by gas chromatography with a flame ionization detector (Lalman and Bagley, 2000). The effective detection limits ranged from 1 mg l^{-1} (in the bottle) for C8–C16 acids to 2 mg l^{-1} for stearic and oleic acids.

Head space gas samples (20 µl) were removed and analyzed via gas chromatography with a thermal conductivity detector to provide hydrogen and methane detection limits of 0.0628 and 0.0486 kPa, respectively. TSS, VSS, alkalinity and pH were determined according to *Standard Methods* (APHA, 1992). The pH for all cultures was 7.4–7.6 with alkalinity of approximately 4800 mg l^{-1} (as $CaCO_3$).

RESULTS

Degradation of oleic and stearic acids

Oleic acid added to cultures was degraded under anaerobic conditions at 21°C (Fig. 1) and within 30

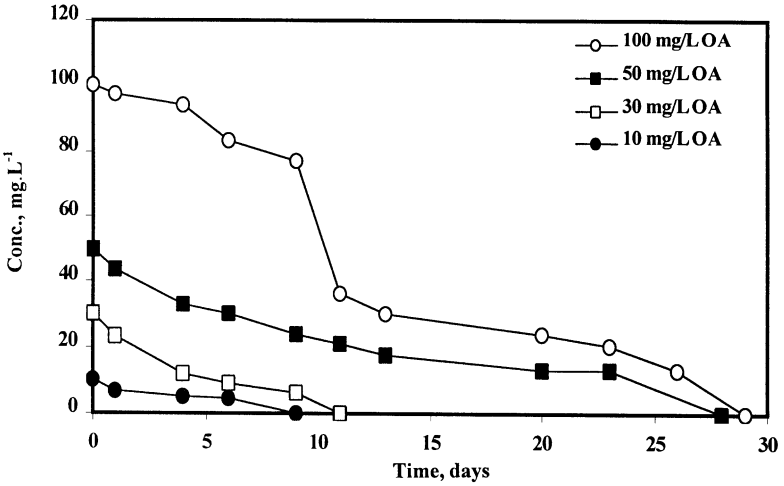

Fig. 1. Oleic acid removal in cultures receiving oleic acid (OA = oleic acid).

Table 1. Maximum concentration of by-products formed during oleic acid degradation

Product formed	Initial oleic acid concentration (μM)[a]			
	36	108	179	357
	Maximum by-product concentration (μM)[b]			
Stearic (C18 : 0) acid	ND[c]	ND[c]	ND[c]	ND[c]
Palmitic (C16 : 0) acid	ND[c]	24 ± 10	115 ± 8	289 ± 21
Myristic (C14 : 0) acid	ND[c]	8 ± 5	42 ± 1	46 ± 1
Acetic (C2) acid	ND[c]	< 8	< 8	485 ± 20

[a] 36 μM = 10 mg L^{-1}, 108 μM = 30 mg L^{-1}, 179 μM = 50 mg L^{-1}, 357 μM = 100 mg L^{-1}.
[b] Average ± S.D. for triplicate bottles.
[c] Not detected.

days it was not detected. Saturated LCFA by-products palmitic and myristic acids were observed in cultures receiving 30, 50 and 100 mg l^{-1} oleic acid but stearic acid was not detected (Table 1). In cultures receiving 30 and 50 mg l^{-1} oleic acid, trace amounts of acetic acid were detected but in cultures receiving 100 mg l^{-1} oleic acid, acetic acid transiently accumulated to a maximum concentration of 29 mg l^{-1} (485 μM) before being degraded within 45 days.

The LCFA by-product distribution, as indicated by the maximum concentration detected, varied with initial oleic acid concentration. The fraction of palmitic acid detected increased from 0% (molar basis) to 22, 64 and 81% as the initial oleic acid concentrations increased from 10 to 30, 50 and 100 mg l^{-1} (from 36 to 108, 179 and 357 μM), respectively. The fraction of myristic acid detected also varied, but not in a systematic manner.

Stearic acid removal occurred much more slowly, with well over 50% remaining in all cultures examined after more than 50 days (Fig. 2). No LCFA by-products were observed, however, acetate was detected near the detection limit in all cultures receiving stearic acid but did not accumulate (data not shown).

Acetic acid inhibition studies

Acetic acid degradation in the presence and absence of diethyl ether is shown in Fig. 3(A). In the presence of 2.0 ml diethyl ether (14.2 g l^{-1} in the culture), the maximum acetic acid degradation rate was retarded from 36 μg acetic acid mg VSS^{-1} d^{-1} to 5.5 μg acetic acid mg VSS^{-1} d^{-1}, and complete acetic acid consumption was accomplished within approximately 14 days. Methane was produced in cultures fed acetic acid in the absence and presence of diethyl ether (Fig. 3(B)).

Oleic acid appeared to inhibit acetic acid consumption at all concentrations examined (Fig. 4(A)). However, the methane production results (Fig. 4(B)) indicate that inhibition due to 10 mg l^{-1} of oleic acid was minimal. The degradation of oleic acid (data not shown but similar to Fig. 1) produced acetic acid at about the same rate it was consumed until day 8, resulting in the constant acetic acid concentration shown in Fig. 4(A). At 30, 50, and 100 mg l^{-1} oleic acid, however, acetic acid consumption was more severely inhibited. Acetic acid accumulated (Fig. 4(A)) and methane production was reduced (Fig. 4(B)).

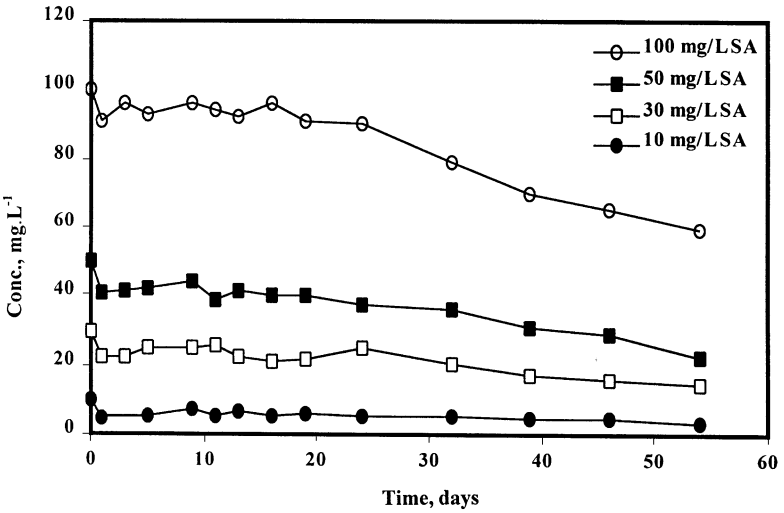

Fig. 2. Stearic acid removal in cultures receiving stearic acid (SA = stearic acid).

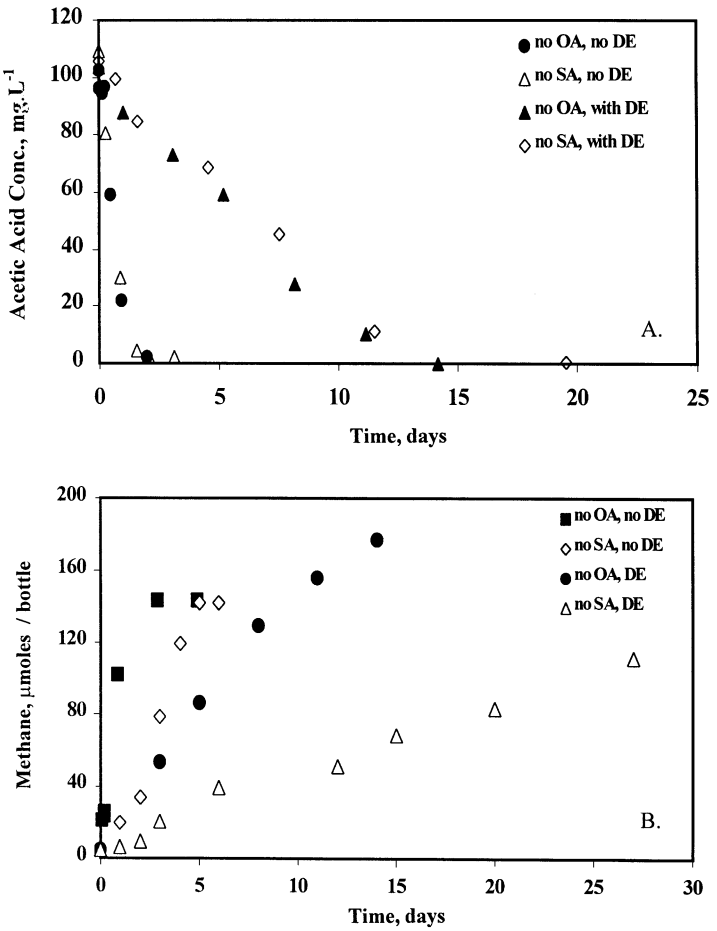

Fig. 3. (A) Acetic acid removal in the absence of oleic and stearic acids. (B) Methane production in the absence of oleic and stearic acids (Ac = acetate, DE = diethyl ether).

Stearic acid appeared to slightly inhibit acetic acid consumption at all concentrations examined (Fig. 5(A)). However, methane production was equal in all cultures (Fig. 5(B)), indicating little inhibition. Stearic acid was approximately 20% removed during the duration of the study. Acetic acid did not accumulate in bottles receiving stearic acid only, suggesting that the acetic acid removal rate in those

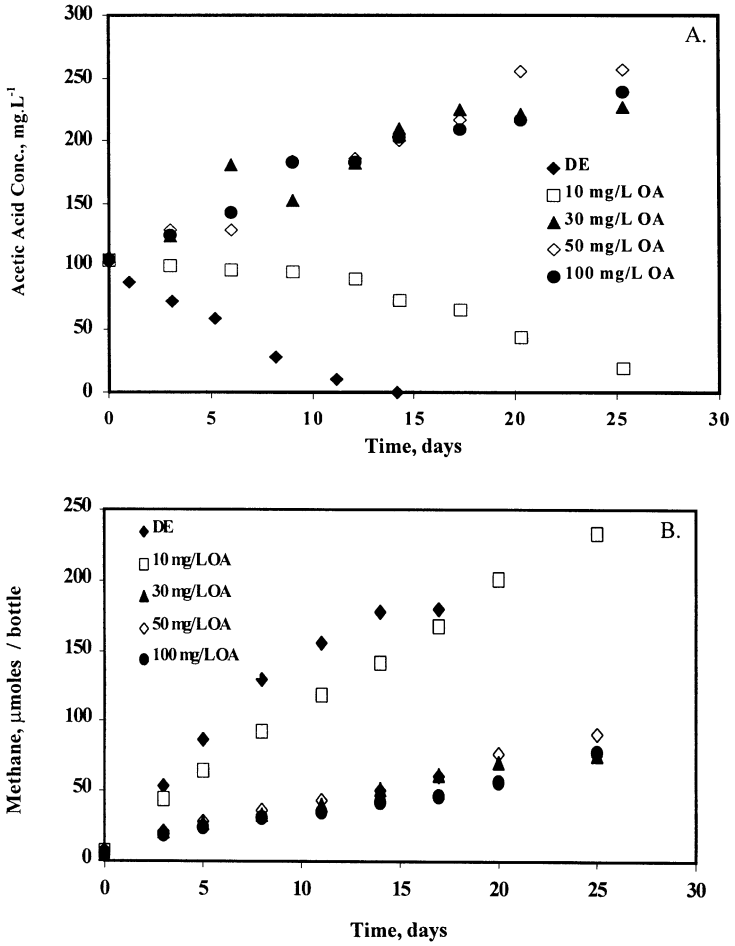

Fig. 4. (A) Acetic acid removal in the presence of oleic acid. (B) Methane production in the presence of oleic acid.

bottles equaled its production rate from stearic acid. Therefore, the apparent discrepancy in bottles that started with 100 mg l^{-1} of acetic acid (Fig. 5(A)) was likely due to the production of acetic acid from stearic acid during the experiment.

Hydrogen inhibition studies

Hydrogen consumption was examined in the presence and absence of diethyl ether and in the presence of different concentrations of either oleic acid or stearic acid. All cultures received approximately 250 µmol of hydrogen and in all cases, the hydrogen was removed within 14 h. Methane production was stoichiometric at all conditions examined (data not shown). During that time no LCFA by-products were detected in the cultures. Oleic acid and stearic acid removals were similar to those shown in Figs 1 and 2.

The hydrogen removal curves for cultures receiving oleic and stearic acids were not linear but followed a first-order expression for all the concentrations examined. Therefore, to provide a means to compare removal rates, the data sets were fit to the general first-order expression $d[H_2]/dt = -k[H_2]$ and the values of k were estimated. Although least-squares regression provided r^2 values >0.96 for all data sets examined, no implication of a kinetic mechanism should be drawn.

The first-order rate coefficients for hydrogen removal are shown in Table 2. The presence of diethyl ether did not impact hydrogen removal rates. The first-order rate coefficients for all the oleic acid concentrations examined were compared using the Tukey's paired comparison procedure (Box *et al.*, 1978). Rate coefficients for cultures receiving 30, 50 and 100 mg l^{-1} oleic acid were not significantly different from each other but were statistically different (95% confidence) from those receiving 10 mg l^{-1}. Furthermore, the cultures receiving 10 mg l^{-1} oleic acid showed significantly lower rate coefficients compared to the pooled controls.

The Tukey's procedure indicated that there were no significant differences between the first-order rate coefficients for any of the stearic acid concentrations examined. However, this may be due to the relatively

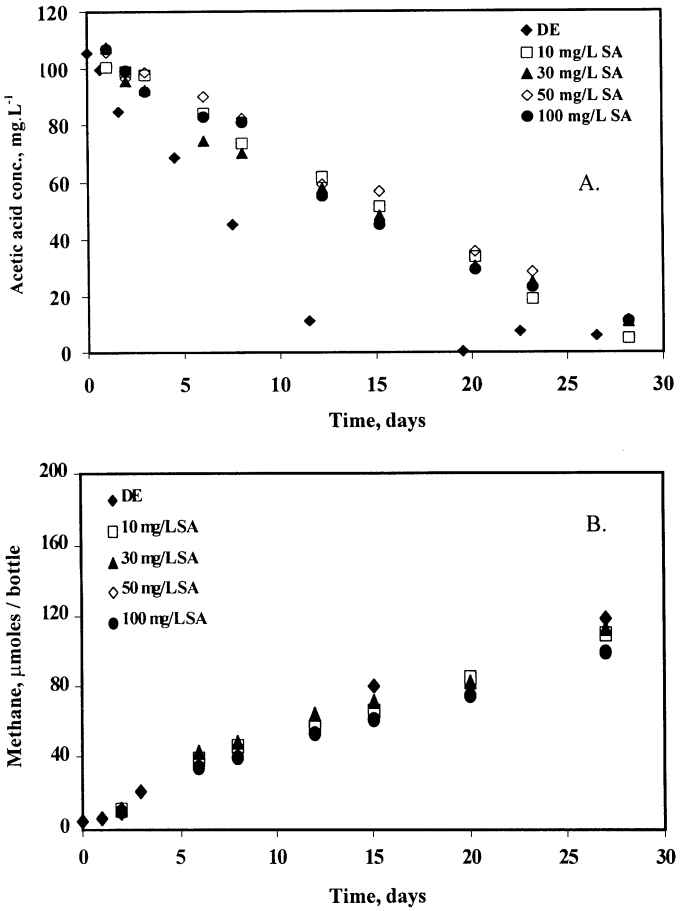

Fig. 5. (A) Acetic acid removal in the presence of stearic acid. (B) Methane production in the presence of stearic acid.

high standard deviations for the 10 and 30 mg l^{-1} conditions as the cultures receiving 50 and 100 mg l^{-1} stearic acid appear to have lower rate coefficients. Nevertheless, the minimum average rate coefficient for the cultures receiving stearic acid was 89% of the control value while for cultures receiving oleic acid, the minimum average rate coefficient was only 70% of the control value.

DISCUSSION

Degradation of C18 fatty acids

The β-oxidation reaction for LCFAs is

$$C_nH_{2n}O_2+2H_2O \rightarrow C_{n-2}H_{2n-4}O_2 + C_2H_4O_2+4e^-+4H^+ \quad (1)$$

The products of each β-oxidation cycle are acetic acid, an *n*-2 LCFA, 4 electrons, and 4 hydrogen ions. The electrons must be moved from cell electron carriers such as FADH and NADH to electron acceptors. Under methanogenic conditions, the ultimate electron acceptor for electrons will be carbon dioxide (producing methane). However, methanogenic organisms do not degrade LCFAs directly. The LCFA-degrading organisms must deposit electrons to an intermediate electron acceptor which can be then used by other organisms as an electron donor. For example, the LCFA-degrading organisms may deposit electrons onto hydrogen which could then be used by hydrogenotrophic methanogenic organisms to produce methane.

Equation (1) describes the β-oxidation of a completely saturated LCFA such as stearic acid. Early researchers proposed that the degradation of unsaturated LCFAs, such as linoleic (C18 : 2) and oleic (C18 : 1) acids, proceeded via β-oxidation only after the unsaturated LCFAs were saturated (Novak and Carlson, 1970). However, although oleic acid was recently observed as a transient product of linoleic acid degradation (Lalman and Bagley, 2000), stearic acid has not been observed as a product of either oleic acid or linoleic acid degradation (Canovas-Diaz *et al.*, 1991; Lalman and Bagley, 2000; this work).

The detection of palmitoleic (C16 : 1) acid as a product of linoleic acid (Lalman and Bagley, 2000) and oleic acid (Canovas-Diaz *et al.*, 1991) provides

Table 2. First-order rate coefficients for hydrogen removal

LCFA concentration ($mg\,l^{-1}$)	First-order rate coefficient (h^{-1})[a]	
	Oleic acid	Stearic acid
0 (without DE)	0.300	0.385
0 (with DE)	0.295	0.381
10	0.262 ± 0.004	0.378 ± 0.012
30	0.222 ± 0.008	0.362 ± 0.017
50	0.209 ± 0.001	0.341 ± 0.008
100	0.212 ± 0.003	0.342 ± 0.004

[a] Average ± SD for triplicate samples except controls which were duplicates.

Table 3. Free energy values[a] for selected reactions involving LCFAs

Parent	Products	Reactions	$\Delta G^{\circ\prime}$ ($kJ\,mol^{-1}$)
Linoleic acid (C18 : 2)	Oleic acid	$C_{18}H_{31}O_2^- + H_2 \rightarrow C_{18}H_{33}O_2^-$	−78.6
	Palmitoleic acid	$C_{18}H_{31}O_2^- + 2H_2O \rightarrow C_{16}H_{29}O_2^- + C_2H_3O_2^- + H_2 + H^+$	−28.1
	Palmitic acid	$C_{18}H_{31}O_2^- + 2H_2O \rightarrow C_{16}H_{31}O_2^- + C_2H_3O_2^- + H^+$	−106.4
Oleic acid (C18 : 1)	Stearic acid	$C_{18}H_{33}O_2^- + H_2 \rightarrow C_{18}H_{35}O_2^-$	−78.6
	Palmitoleic acid	$C_{18}H_{33}O_2^- + 2H_2O \rightarrow C_{16}H_{29}O_2^- + C_2H_3O_2^- + 2H_2 + H^+$	50.5
	Palmitic acid	$C_{18}H_{33}O_2^- + 2H_2O \rightarrow C_{16}H_{31}O_2^- + C_2H_3O_2^- + H_2 + H^+$	−27.8
Stearic acid (C18 : 0)	Palmitic acid	$C_{18}H_{35}O_2^- + 2H_2O \rightarrow C_{16}H_{31}O_2^- + C_2H_3O_2^- + 2H_2 + H^+$	50.8
Palmitoleic acid (C16 : 1)	Palmitic acid	$C_{16}H_{29}O_2^- + H_2 \rightarrow C_{16}H_{31}O_2^-$	−78.3
	Myristic acid	$C_{16}H_{29}O_2^- + 2H_2O \rightarrow C_{14}H_{27}O_2^- + C_2H_3O_2^- + H_2 + H^+$	−31.8
Palmitic acid (C16 : 0)	Myristic acid	$C_{16}H_{31}O_2^- + 2H_2O \rightarrow C_{14}H_{27}O_2^- + C_2H_3O_2^- + 2H_2 + H^+$	46.5
Myristic acid (C14 : 0)	Lauric acid (C12 : 0)	$C_{14}H_{27}O_2^- + 2H_2O \rightarrow C_{12}H_{23}O_2^- + C_2H_3O_2^- + 2H_2 + H^+$	48.6

[a] Standard free energies at 25°C and activities = 1 except for H^+ activity = 10^{-7} (pH = 7). See Lalman (2000) for calculation details.

further evidence that LCFAs need not be completely saturated prior to β-oxidation. Furthermore, palmitic and myristic acids were produced relatively rapidly during the degradation of linoleic and oleic acids (Lalman and Bagley, 2000; Canovas-Diaz *et al.*, 1991; this work) but no LCFA by-products were detected during the degradation of stearic acid (Angelidaki and Ahring, 1995; this work). The detection of saturated *n*-2 LCFAs from unsaturated parent LCFAs does not prove that stearic acid was not first formed, but in conjunction with the much slower rate of stearic acid degradation compared to oleic acid degradation (see Figs 1 and 2), this hypothesis appears reasonable.

A possible explanation for the hypothesis that unsaturated LCFAs need not be saturated prior to β-oxidation arises from a consideration of the free energies involved. The free energies for several relevant reactions are shown in Table 3. For comparative purposes, the β-oxidation reactions are shown to produce hydrogen. The free energies are at standard conditions of 25°C, 1 atm and activities of 1 except for hydrogen ion (pH) which has been corrected to an activity of 10^{-7} (pH = 7). Those reactions with positive free energies may still occur under appropriate environmental conditions, for example, with hydrogen partial pressures on the order of 5×10^{-5} atm instead of 1 atm.

The products of C18 LCFA degradation are also shown in Fig. 6 on the basis of their relative reaction energies. For example, energy is released when linoleic acid is reduced to oleic acid but energy is required when stearic acid is β-oxidized to palmitic acid, acetic acid and hydrogen. The products at the end of the unmarked arrows have been experimentally detected and are energetically favored versus the reactants. The products at the end of arrows marked with ? have been detected but the pathway is either energetically unfavorable at standard conditions or otherwise uncertain. The products at the end of arrows marked with *X* have not been detected experimentally, although they may be energetically favorable.

The production of palmitic acid from linoleic and oleic acids is energetically favorable but its production from stearic acid is not. Lowering the hydrogen partial pressure to facilitate the stearic acid β-oxidation reaction makes the oleic acid conversion to palmitic acid more energetically favorable as well. Similarly, the production of myristic acid from linoleic acid is energetically favorable, but its production from oleic acid and stearic acid, in particular, is not. The production of lauric (C12 : 0) acid from linoleic acid is somewhat favorable ($\Delta G^{\circ\prime} = -11.3\,kJ\,mol^{-1}$), but the production of C10 and lower fatty acids from C18 acids becomes energetically unfavorable at standard conditions for all three acids.

These energetic predictions of product distribution were observed experimentally with palmitic and myristic acids being the primary detected intermediates from linoleic acid degradation (Lalman and Bagley, 2000), palmitic acid being the primary detected product from oleic acid degradation (this work) and no LCFA products detected from stearic acid (this work). That intermediates were detected at

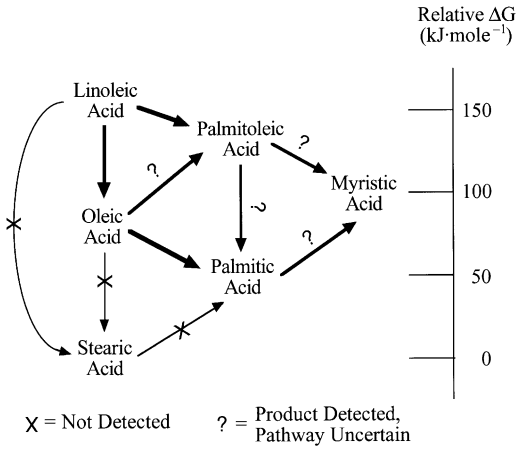

Fig. 6. Degradation pathways for C18 LCFAs.

all suggests that the parent LCFAs were more competitive for the reaction site than the intermediates. If stearic acid was formed from linoleic or oleic acid reduction (reactions that are actually energetically favorable, Table 3), it would be expected to transiently accumulate until environmental conditions changed sufficiently to allow its degradation. This was not observed, indicating that β-oxidation of unsaturated C18 LCFAs is a likely pathway to initiate degradation of these compounds.

Inhibition of methanogenesis by oleic and stearic acids

The addition of oleic acid inhibited aceticlastic methanogenesis (Figs. 4(A) and (B)). The diethyl ether used to deliver the oleic acid also caused inhibition of aceticlastic methanogenesis. However, the added diethyl ether concentration was more than 140 times that of the largest oleic acid concentration and acetic acid removal was still detected (Fig. 3(A)). When as little as $30\,mg\,l^{-1}$ oleic acid was added, acetic acid removal (and concurrent methane production) occurred at a reduced rate versus the diethyl ether control. The inhibition of aceticlastic methanogenesis by oleic acid was previously observed in systems operating at 55°C (Angelidaki and Ahring, 1992). In that case inhibition was not observed until more than than $100\,mg\,l^{-1}$ oleic acid was added.

The addition of stearic acid did not appear to significantly inhibit aceticlastic methanogenesis at the concentrations examined in this study. Angelidaki and Ahring (1992) observed inhibition of aceticlastic methanogenesis at 55°C due to stearic acid but only when more than $300\,mg\,l^{-1}$ were added.

The addition of diethyl ether showed no inhibition of hydrogenotrophic methanogenesis. Oleic and stearic acid concentrations above $30\,mg\,l^{-1}$ caused only slight inhibition of hydrogenotrophic methanogenesis. These results are consistent with those reported for linoleic acid (Lalman and Bagley, 2000) and suggest that at the concentrations examined, the inhibition of aceticlastic methanogenesis in systems treating vegetable oils would be more of a concern than the inhibition of hydrogenotrophic methanogenesis.

The biomass used for these experiments was enriched using glucose in the absence of oleic or stearic acids. In the continuous presence of oleic acid, stearic acid or other LCFAs, acclimation may occur, reducing the inhibitory effects. If so, the relevant concern becomes determining the maximum LCFA concentration that can be withstood by the system, a concern that must be answered experimentally on a case by case basis.

CONCLUSIONS

Oleic and stearic acids are major constituents of vegetable oils but their anaerobic degradability and inhibitory properties at concentrations less than $100\,mg\,l^{-1}$ and at a temperature near 20°C have not been examined. The conclusions from this study are:

1. Oleic acid was anaerobically degraded at 21°C but stearic acid degradation was very slow in unacclimated cultures.
2. Saturated C16 and C14 by-products transiently accumulated during oleic acid degradation but no LCFA by-products were detected from stearic acid degradation.
3. Oleic acid at concentrations above $30\,mg\,l^{-1}$ inhibited aceticlastic methanogenesis at 21°C but stearic acid did not inhibit acetic acid consumption at the concentrations examined.
4. Oleic acid and stearic acid slightly inhibited hydrogenotrophic methanogenesis at 21°C.
5. The production of shorter chain LCFAs from oleic and linoleic acid is more energetically favorable than from stearic acid, supporting the hypothesis that the β-oxidation of unsaturated C18 LCFAs can occur directly.

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REFERENCES

Angelidaki I. and Ahring B. K. (1992) Effects of free long chain fatty acids on thermophilic anaerobic digestion. *Appl. Microbiol. Biotechnol.* **37**, 808–812.

Angelidaki I. and Ahring B. K. (1995) Establishment and characterization of an anaerobic thermophilic (55°C) enrichment culture degrading long-chain fatty acids. *Appl. Environ. Microbiol.* **61**, 2442–2445.

APHA, AWWA, WPCF (1992) *Standard Methods for the Examination of Water and Wastewater*. 18th ed., American Public Health Association, Washington, DC 20005.

Box G. E. P., Hunter W. G. and Hunter J. S. (1978) *Statistics for Experimenters: an Introduction to Design, Data Analysis and Model Building*. Wiley, Toronto.

Canovas-Diaz M., Sanchez-Roig M. J. and Iborra J. L (1991) Myristic and oleic acid degradation by an acclimated anaerobic consortia: synergistic behavior. In *Biomass for Energy, Industry and Environment. 6th E.C. Conference*, eds G. Grassi, A. Collina and H. Zibetta, pp. 580–584. Elsevier Applied Science, London, UK.

Demeyer D. I. and Hendrickx H. K. (1967) The effect of C_{18} unsaturated fatty acids on methane production in vitro by mixed rumen bacteria. *Biochim. Biophys. Acta* **137**, 484–497.

Hanaki K., Matsuo T. and Nagase M. (1981) Mechanism of inhibition caused by long chain fatty acids in anaerobic digestion. *Biotechnol. Bioengng.* **27**, 1591–1610.

Hickey R. F., Vanderwielen J. and Switzenbaum M. S. (1987) The effects of organic toxicants on methane production and hydrogen gas levels during the anaerobic digestion of waste activated sludge. *Water Res.* **21**(11), 1417–1427.

Johns M. R. (1995) Developments in wastewater treatment in the meat processing industry: A review. *Biores. Technol.* **54**, 203–216.

Koster I. W. and Cramer A. (1987) Inhibition of methanogenesis from acetate in granular sludge by long chain fatty acids. *Appl. Environ. Microbiol.* **53**(2), 403–409.

Lalman J. A. (2000) Anaerobic degradation of Linoleic (C18 : 2), Oleic (C18 : 1) and Stearic (C18 : 0) acids and their inhibitory effects on acidogens, acetogens and methanogens. Ph.D. Thesis, Department of Civil Engineering, University of Toronto, Tor. Ont., Canada.

Lalman J. A. and Bagley D. M. (2000) Anaerobic degradation and inhibitory effects of linoleic acid. *Water Res.* **34**(17), 4220–4228.

Novak J. T. and Carlson D. A. (1970) The kinetics of anaerobic long chain fatty acid degradation. *J. Water Pollut. Cont. Fed.* **42**, 1932–1943.

O'Brien R. D. (1998) *Fats and Oils: Formulation and Processing for Applications*. Chapter 1. Technomic Publishing Co., Inc. Lancanster, PA.

O'Rourke J. T. (1968) Kinetics and anaerobic waste treatment at reduced temperatures. Ph.D. Dissertation, Department of Civil Engineering, Stanford University, Stanford, CA, USA.

Ralston A. W. and Hoerr C. W. (1942) The solubilities of the normal saturated fatty acids. *J. Organic Chem.* **7**, 546–555.

Sonntag N. O. V. (1979) Composition and characteristics of individual fats and oils. In *Bailey's Industrial Oil and Fat Products*, ed. D. Swern, 4th ed. Chapters 5 and 6. Wiley, New York, NY.