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Design and preliminary results of an NMR tube reactor to study the oxidative degradation of Fatty Acid Methyl Ester

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Abstract

Biodiesel is the fatty acid alkyl esters produced by the transesterification of vegetable, animal or microbial lipids. After ethanol, it accounts for the largest proportion of global biofuel production. Yet, due to the level of polyunsaturation, biodiesel is also oxidatively unstable. When biodiesel oxidizes the viscosity increases, which leads to reduced fuel performance and in extreme cases can lead to engine failure. To aid in understanding the process of this degradation a specialist NMR tube rig was designed to assess the oxidation of biodiesel. The NMR tube rig allowed the *in situ* ^{1}H NMR measurement of the sample while air was bubbled through at fixed intervals. The methyl esters of linolenic acid (18:3), linoleic acid (18:2) and oleic acid (18:1) were oxidised at 110 °C over a 24 hour period. The decomposition of biodiesel is complex, and there is more than one mechanism involved in the degradation. Using this rig the onset of oxidation for 18:3 and 18:2 was found to be almost instantaneous. The rate of oxidation was found to be slightly less for 18:2 than 18:3

while the maximum rate was observed for 18:3 from the beginning of the oxidation, this was only observed after 280 mins for 18:2. The oxidation of 18:1 started at approximately 500 minutes and, slowly degraded during the remaining reaction time. The formation of a number of secondary oxidation products such as aldehydes, ketones, alcohols and formates were also quantified.

1 Introduction

Due to the increasing pressure to reduce greenhouse gases and the growing scarcity of fossil fuels, alternatives to liquid transport fuels are being increasingly sort. One such fuel is biodiesel, the fatty acid alkyl esters (FAAE) derived from the transesterification of vegetable, waste, animal or microbial lipids. Biodiesel has comparable properties to diesel fuel and, as such, can be used as a substitute for petroleum diesel in compression ignition engines. However, biodiesel is generally high in polyunsaturated esters which are prone to oxidation. As the fuel degrades volatile acids are given off, the viscosity increases substantially and eventually solid particles and gums are formed [1]. This is problematic as the oxidised fuel can block filters and injectors as well as leading to poor combustion and an increase in toxic emissions such as particulate matter (PM), polyaromatic hydrocarbons (PAH), formaldehyde, acetaldehyde and acrolein [2]. This problem is further exacerbated on modern vehicles equipped with a diesel particulate filter (DPF) where the fuel will slowly accumulate in the engine sump oil during filter regeneration events. While petroleum diesel will generally evaporate at the normal working temperature of the lubricating oil, and be recirculated back into the engine intake air for combustion, the biodiesel will persist and degrade, necessitating a premature oil change [3].

The rate of oxidation of biodiesel is dependent on many factors including the air available, metal content, temperature, light and most importantly the fatty acid profile [4, 5]. The most common fatty acids found in biodiesel are palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2) and linolenic acid (18:3). The oxidative stability increases with increasing saturation and, as such, biodiesel rich in saturated esters such as palm or coconut oil methyl ester containing palmitic acid, tend to be more stable than those rich in polyunsaturates such as soybean oil methyl ester which is predominantly made up of linoleic and linolenic acids [6].

The oxidation of unsaturated lipids has been extensively researched previously with studies detailing the reaction of triglycerides and related compounds, the effect of the oxidation of lipids on appearance, flavor and toxicity as well as the role of natural anti-oxidants in these processes [7, 8]. More recently research has primarily been focused on detailing the oxidation of biodiesel [9]. From these studies FT-IR spectroscopy [10, 11], UV-VIS spectroscopy [12], GC-MS [13], peroxide or acid value and NMR spectroscopy have all been used to assess the decomposition [14, 15]. Though a number of mechanisms have been proposed detailing how biodiesel can degrade, it seems clear that the primary mechanism, the auto-oxidation, proceeds with the formation of a radical hydrocarbon species on the bisallylic carbon. This is followed by isomerization into a more stable structure. This radical will then react with oxygen in the air to form a peroxide species which further propagates the reaction. The peroxides can then break down into oxygenated intermediates which further degrade into small chain acids, ketones, alkenes and aldehydes [16]. The peroxide species' can also form dimers and oligomers [17]. However, further work has shown that an oligomeric peroxide species, with more than one peroxide linker, can also be formed which propagates the reaction by rapidly breaking down into aldehydes, acids and an alkoxy radical species [18]. These aldehydes are unsaturated if the oxidised chains are joined by multiple peroxide linkers [19]. NMR spectroscopy has the potential to provide significant new information on biodiesel oxidation, but the few studies utilizing this technique that have been reported so far have used bench-top sampling techniques rather than *in situ* NMR experiments. Therefore, to further investigate the products and kinetics in the oxidation of biodiesel, a rig was designed to allow the *in situ* analysis of the oxidation of pure FAME samples via ^{1}H NMR spectroscopy.

2 Experimental

2.1 Materials

Deuterated xylene and dodecane were purchased from Fluorochem, oleic acid methyl ester, 18:1, (99.0%), linoleic acid methyl ester, 18:2, (99.0%) and linolenic acid methyl ester, 18:3, (99.0%) were purchased from Sigma-Aldrich and used without further purification. All chemicals were stored at -85 °C prior to use.

2.2 ^{1}H NMR spectroscopy method

The NMR rig was created using standard silicone tubing, glass and polycarbonate casing with Teflon seals. NMR spectroscopic measurements were carried out at 383 K using a Bruker AV500 spectrometer, operating at 500.13 MHz for ^{1}H. Standard Bruker pulse sequences were used throughout. ^{1}H spectra were typically acquired using a 30 degree excitation pulse and a repetition time of 4.2 sec. 1.0 Hz line broadening was applied before Fourier transform, and spectra were referenced to the residual xylene solvent peak (δ 6.90 ppm).

In a typical run, FAME (0.05 ml) was added to deuterated xylene (0.9 ml) and deuterated dodecane (0.05 ml) in the NMR tube rig, which was held statically in the NMR machine. The temperature of the NMR machine was set to 110 °C and allowed to stabilize. The NMR rig was then inserted into the NMR machine, the sample was shimmed and an NMR spectrum was recorded. This was taken as time = 0. Air was bubbled through the tube at a rate of 1 ml min^{-1} over a period of 8 minutes using a peristaltic pump with automated control settings, after which the mixture was allowed to settle for 2 mins. During this time, a ^{1}H NMR spectra was recorded. This process was repeated over a 24 hour period, with both the pump and the data acquisition under automated control. After processing, the spectra were integrated relative to the residual solvent peak of the xylene which was found to differ by less than 3.5% throughout the course of the reaction. Three replicates of the reaction were run to assure the consistency of the data.

To aid in the assignment of the oxygenated products, COSY, HSQC and HMBC experiments were undertaken on a sample of 18:2 after 500 min reaction time. The sample was oxidized under the conditions described above, the sample was then crash cooled to halt the oxidation and redissolved in $CDCl_3$ prior to analysis.

3 Results and Discussion

3.1 Reactor Design

In the oxidation of biodiesel, small chain organic acids are produced in significant quantities [20], therefore, the NMR tube rig was designed to be air-tight with a stable outlet to remove these volatiles from the reaction mixture. The tube reactor had not only to be acid resistant, but also stable at 110 °C. Glass, treated silicone tubing, teflon and polycarbonate were considered suitable materials. The reactor consisted of a standard glass NMR tube with screw top, a sealed polycarbonate casing was built around this allowing an air-tight inlet and outlet section fitted with silicone piping (Fig. 1).

As the silicone material has the potential to swell if infused with solvent, which would effectively seal the tube, a long glass rod was attached to the inlet. Solid materials such as the glass rod, as well as gas bubbles, degrade the homogeneity of the magnetic field and results in severe line broadening making quantification of the individual peaks difficult. One method to overcome this was to hold the inlet pipe above the region where the measurement was made, however, this technique resulted in poor mixing and a low interaction between the air and biodiesel. Instead the glass rod was held symmetrically in the centre of the tube by pushing it against the bottom. This had the added benefit of dispersing the gas bubbles and increasing the surface interaction between the two phases. This concentric tube arrangement gives reasonably good magnetic homogeneity and, without bubbling, the resulting spectra had acceptable NMR lineshapes. To reduce line broadening due to the gas bubbles, the air at 1 ml min^{-1}, was pumped through the system using a peristaltic pump running an automated program with 2 minutes of down time every 10 minutes. This was shown to be sufficient to allow the mixture to settle and an NMR spectra to be recorded. The NMR spectra were recorded in a mixture of deuterated dodecane (5%) and xylene (95%), as it was thought this solvent mixture was a reasonable (cost permitting) model for diesel or engine oil fuel.

3.2 Oxidation of pure FAME samples

The main oxidative mechanism, the auto-oxidation, proceeds by the formation of a carbon radical on the bisallylic carbon, the double bonds then conjugate, to form a more stable system and the radical reacts with oxygen to form a peroxide species [7]. As there is no bisallylic site on 18:1, this mechanism is less favoured and other oxidative mechanisms can also be observed such as the formation of an epoxide [21]. The signal relating to unconjugated double bonds (***δ*** 5.3 – 5.5 ppm) can then be used to assess the degradation. Despite the absence of light in the NMR machine, the oxidation proceeds rapidly. 18:3 begins to decompose immediately and by 600 minutes only 20% of the starting material remains (Fig. 2). 18:2 oxidises more slowly than the 18:3, though the onset of oxidation is almost immediate. There is no visible reduction in the double bonds of 18:1 until 500 minutes into the reaction (Fig. 3a). After this point the double bonds slowly reduce. A similar trend is observed for the degradation of the bisallylic sites (Fig. 3b). After 24 hours of oxidation, less than 5% of the original sites remain in both 18:2 and 18:3.

In order to determine the instantaneous and maximum rate of degradation for each FAME type, it was necessary to remove noise from the raw data using filtering. Within the MatLab environment, a zero-phase digital filter was applied in both the forward and reverse directions (Fig. 4). The resultant filtered FAME concentration data was then differentiated to determine the rate of degradation and the maximum rate was identified along with the time at which it occurred. Table 1 summarises the rate data for each FAME type. As can be seen, the rate of decomposition is related to the structure, with 18:3 demonstrating the highest rate occurring almost instantaneously at the start of the test. 18:2 shows a very similar trend to 18:3, but its maximum rate of decomposition is not achieved until 280 minutes into the reaction. It would appear that 18:1 never achieves a maxima, as the rate of decomposition is continuing to increase even after 1430 minutes. Using the NMR reactor the maximum rate of the oxidation of 18:2 and 18:3 is highly similar while the degradation of 18:1 was found to be five times lower.

3.3 Analysis of secondary products

Some of the major secondary products in the oxidation of biodiesel are aldehydes. These components are observed during the decomposition in relatively large quantities for the oxidation of the polyunsaturated FAMEs (Fig. 5). In the oxidation of 18:1, small amounts are observed on degradation of the double bonds and it seems likely that the aldehydes are also a decomposition product from the oxidation of the monounsaturated component. In the degradation of 18:2 and 18:3, aldehydes are produced from the onset, reach a maxima at roughly 500 minutes into the reaction time, and then both reduce slowly over the remaining time.

The reduction in the aldehyde peak corresponds with the decrease in the rate of double bonds being consumed. Aldehydes are a primary decomposition product from the oxidative breakdown of lipids, and previous studies have shown, that on the oxidation of polyunsaturated lipids, a range of unsaturated and saturated aldehydes are formed on the oxidation of the pure lipids [8]. To further assess the applicability of using NMR to aid in the assignment of these oxygenates, COSY, HSQC and HMBC NMR experiments were undertaken (given in the supporting information). COSY spectra show which protons are coupled to each other, and therefore typically on adjacent carbon atoms. Such coupling is indicated by the presence of cross-peaks (off-diagonal peaks) at the chemical shift of one proton on the vertical axis and its coupled proton on the horizontal axis. In the resulting COSY plot it can be seen that a minority of the aldehyde protons at 9.4 – 9.8 ppm are coupled to the protons of saturated carbon chains at 2.4 ppm, whereas the majority are coupled to alkenic protons at 6.1 – 6.4 ppm.

Likewise, HSQC spectra reveal the chemical shift of the carbon atoms directly bonded to each proton. Since ^{13}C chemical shifts are spread out over a greater range than ^{1}H chemical shifts, the ^{13}C shift associated with a particular ^{1}H resonance is often useful as a diagnostic tool. By running an HSQC NMR experiment the majority of the protons were found to couple to carbonyl carbon atoms at around δ 194 ppm further suggesting that the majority of the aldehydes are unsaturated, the rest of the aldehydes coupled to a peak at δ 204 ppm, suggestive of a saturated aldehyde This is supportive that the breakdown of 18:2 follows from the decomposition of carbon chains with multiple peroxide linkers [19]. The oxidation of 18:2 using the NMR reactor produces over twice as many aldehydes than the decomposition of 18:3, this major difference is presumably due to the formation of more volatile components such as crotonaldehyde during the oxidation of 18:3, as opposed to longer less volatile components in the oxidation of 18:2, a mechanism proposed by Frankel and co-workers [8].

On the decomposition of biodiesel, formic acid is also produced [20]. The formic acid is volatile and will evaporate from the reaction mixture. In a previous study conducted on the oxidation of RME, a range of formates, in addition to formic acid, have been observed in the reaction mixture [21]. It was assumed that formic acid was being formed and reacting with alcohols, a further decomposition product in the oxidation. In the NMR tube reactor, only a minor amount of formic acid or formates are observed in the oxidation of 18:1 and 18:3 (Fig. 6). Formic acid is present, but is presumably being removed from the system more efficiently due to the air flow. In the oxidation of 18:2 however, a number of peaks are observed in this region, suggesting that other types of formates are being produced in large quantities solely from the oxidative breakdown of this particular FAME, this assignment is supported up by the lack of coupling to any other protons in the COSY plot.

If a number of formate esters are being formed then the protons on the α-carbon of the ester moiety will be observed between δ 3.7 and 4.5 ppm. Over the course of this study very little is observed in this region for 18:1 or 18:3, however, like the formate proton there are a number of peaks found in this region for 18:2 (Fig. 7). Though protons attributable to ketones or aldehydes will not appear in this region other possibilities are peroxides, the α-protons of an alcohol or the hydroxyl proton itself. It seems unlikely that the hydroxyl protons come in this area as the addition of D_2O did not change the spectra (see supporting information). The HSQC plot demonstrates that the protons are bound to carbons with shifts between δ 68 - 86 ppm, this is consistent with them being attributable to the α-protons of alcohols or formate esters. The COSY plot of this region shows that the majority of these protons are coupling to protons found in the region δ 1.5-1.7 ppm, with the rest coupled to alkenic protons. This suggests that though a majority of this signal corresponds to long chain saturated alcohols and formates, there are still a proportion of oxygenated products with a remaining double bond on the chain.

The oxygenates in this region reach a maxima at around 500 minutes into the reaction, after this point the concentration does not change to any significant degree (Fig. 8). This is not the case in 18:3, suggesting that other products, unique to the breakdown of 18:3, are themselves decomposing over the course of the reaction, but being produced in much lower quantities than for 18:2. For the oxidation of 18:1, the alcohol or formate components are observed as soon as the double bonds start to oxidise and this amount continues to increase throughout the reaction time, suggesting they represent a major decomposition product of the breakdown.

The other primary decomposition products in the oxidation of lipids are ketones [7, 8]. Ketones can be identified by a characteristic shift between δ 2.45 – 2.75 ppm in the proton spectra. Aside from ketones however, ethers and epoxides will all have shifts in this region. However, the HSQC plot shows correlations to carbons at around δ 44 ppm, typical of ketones. Furthermore, the HMBC spectrum, which gives similar information to the HSQC experiment except that it shows coupling from a particular proton to carbons two or three bonds away rather than one bond, shows coupling to carbonyl peaks found at δ 200 and 204 ppm. This demonstrates that though ethers and epoxides can be formed in the oxidation of biodiesel [20, 21], they were not observed under these conditions. The ketones are observed in the decomposition of all the FAMEs analysed over the course of this study (Fig. 9). The protons found in this region coupled entirely to protons observed at δ 1.5 - 1.6 ppm and not to any alkenic protons. This suggests that all the double bonds have reacted when these ketones are formed. The ketones were observed in the same molar percentage as the aldehydes although, unlike the aldehydes, the ketones appear to be a stable product of the oxidation. The oxidation of 18:3 produces the largest integral across this area, noticeably higher than that of 18:2.

4. Conclusions

A specialist NMR rig was designed to further investigate the oxidation of pure FAME samples to further understand the pathways and decomposition products of biodiesel. 18:3 and 18:2 were found to decompose almost instantaneously under the reaction conditions, though the rate of decomposition was at its highest at the beginning of the reaction for 18:3, this was not the case with 18:2. The decomposition of 18:1 was observed to only commence after 500 minutes of the reaction, at its highest the rate of decomposition was still approximately five times slower than for 18:2 or 18:3. In addition a larger quantity of aldehydes, esters, acids and alcohols were observed in the decomposition of 18:2 than in the other reactions, with the presence of these compounds suggesting that the breakdown of 18:3 produces more volatile components whereas 18:2 is breaking down through a number of mechanisms presumably forming oligomeric peroxide species with multiple linkers. All the oxygenates were found to be a mix of saturated and unsaturated for 18:2 and 18:3, with ketones seemingly being the most stable oxidation products.

5. Acknowledgements

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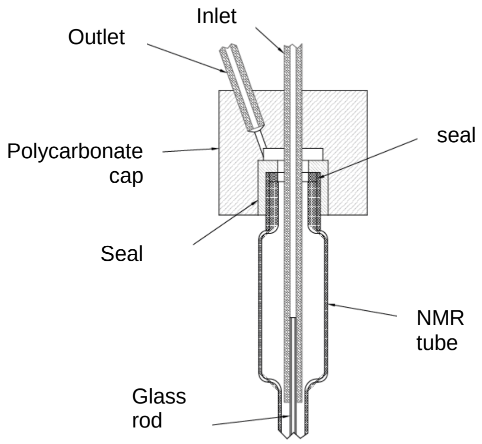

Figure 1. NMR tube reactor design

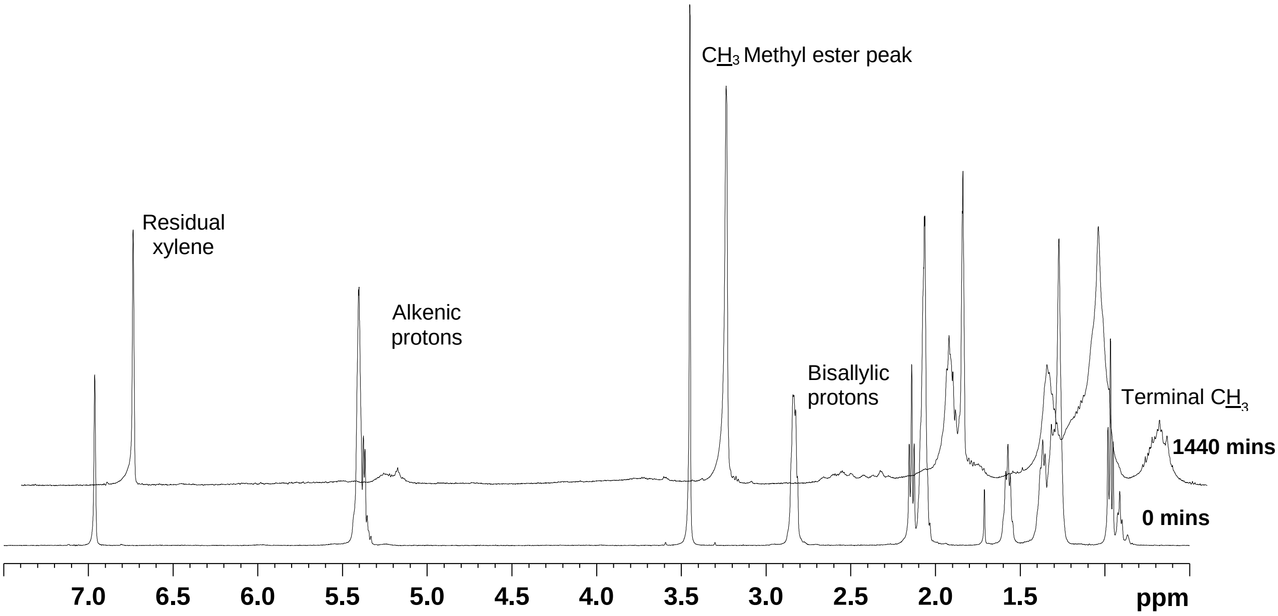

Figure 2. Representative ^{1}H NMR spectra of 18:3, showing the spectra recorded at 0 and 1440 minutes, the upper spectra has been shifted to the right for clarity and is not to scale.

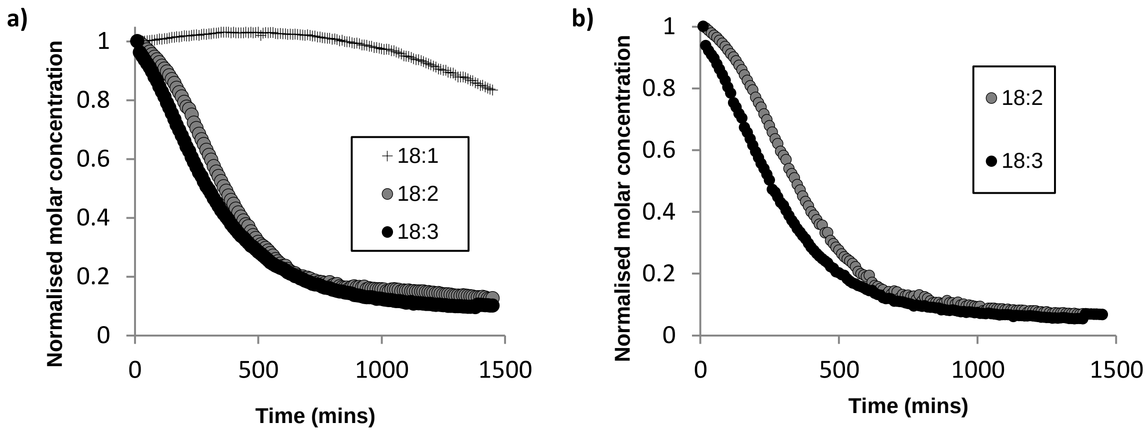

Figure 3. a) Reduction in the double bonds of 18:1, 18:2 and 18:3, b) reduction in the bisallylic protons for 18:2 and 18:3

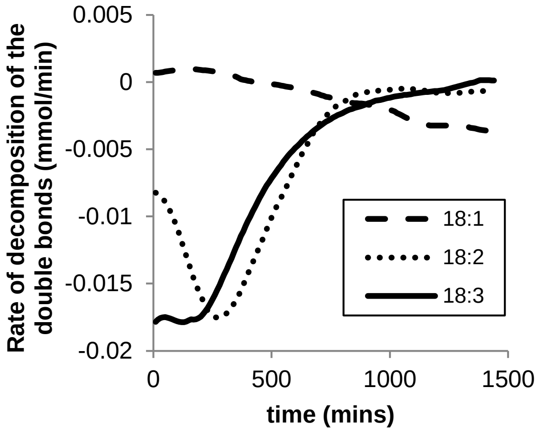

Figure 4 Rate of the consumption of double bonds throughout the reaction, by integration of the peak δ 5.3-5.5 ppm for the pure FAME samples 18:1, 18:2 and 18:3

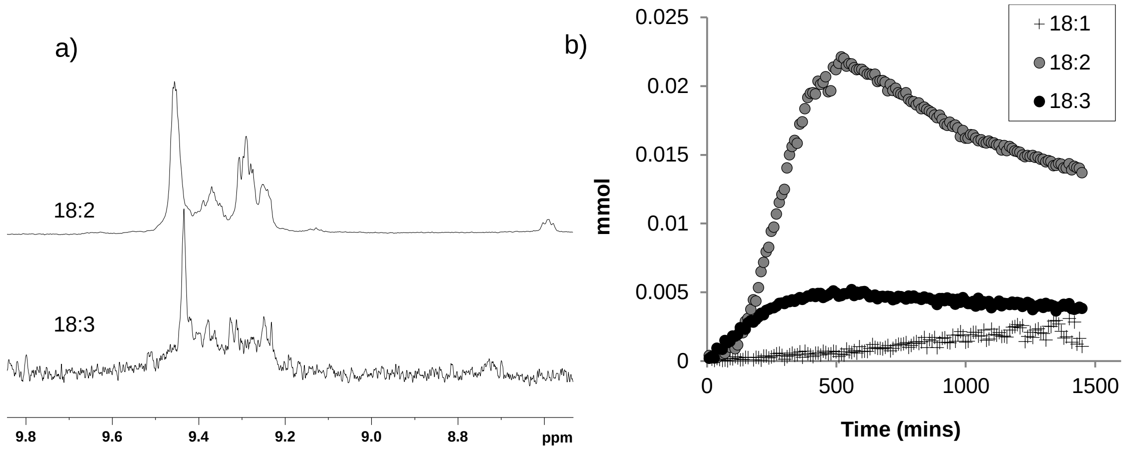

Figure 5 a) Aldehyde region (δ 9.0 – 9.5 ppm) of the ^{1}H NMR spectra after 8 hours oxidation, b) aldehydes produced throughout the course of the reactions for 18:1, 18:2 and 18:3

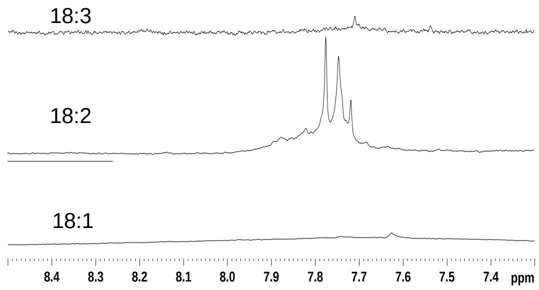

Figure 6. Formate region (δ 7.5 – 8.0 ppm) of the ^{1}H NMR spectra after 24 hours oxidation

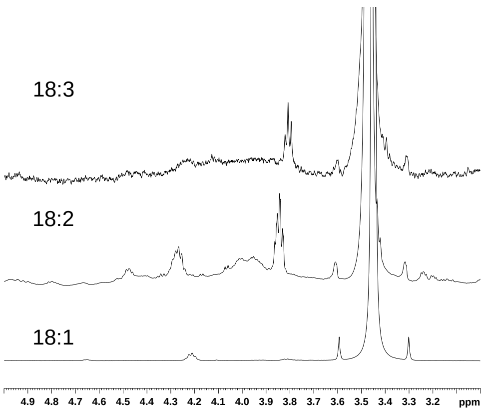

Figure 7 Region in the ^{1}H NMR spectra (δ 3.7-4.5 ppm) attributable to the α-protons on the esters and alcohols formed after 500 minute reaction time

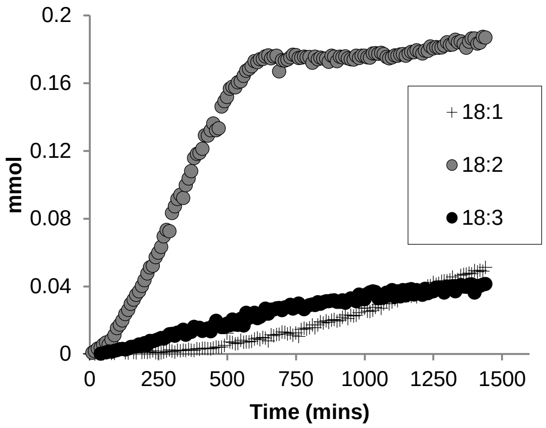

Figure 8. Integral plot of the region δ 3.7 - 4.6 ppm, predominated by the α-protons of esters and alcohols produced throughout the course of the three reactions

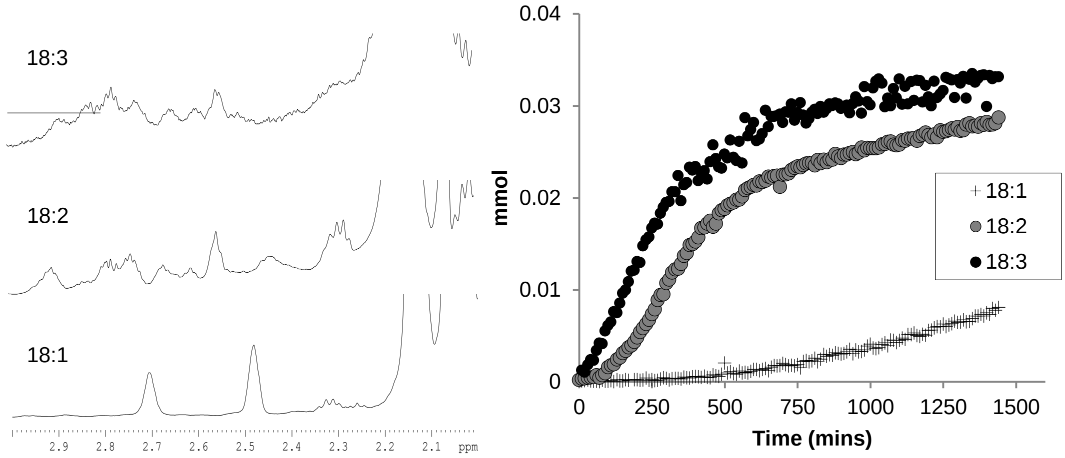

Figure 9. The ketone region (δ 2.45-2.74 ppm) of the a) ^{1}H NMR spectra and b) the integral of the region plotted against time for the three FAME samples

FAME Type	Time of Max. Rate (mins)	Max. Rate (mmol/min)
18:1	**1430**	**-0.0037**
18:2	**280**	**-0.0175**
18:3	**130**	**-0.0179**

Table 1 – Rate of FAME degradation

Supplementary information

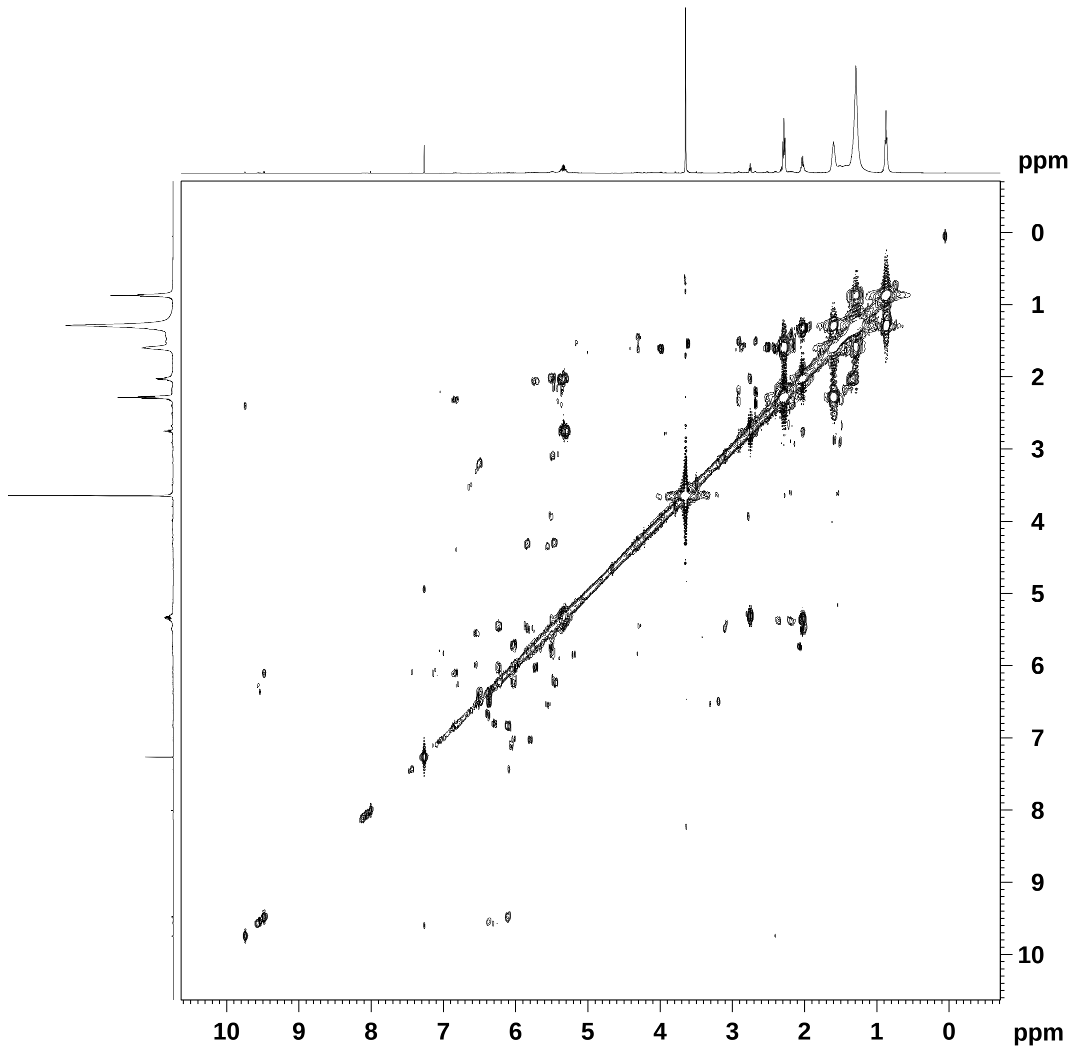

Fig. S1 COSY PLOT of 18:2 after 500 minutes reaction time

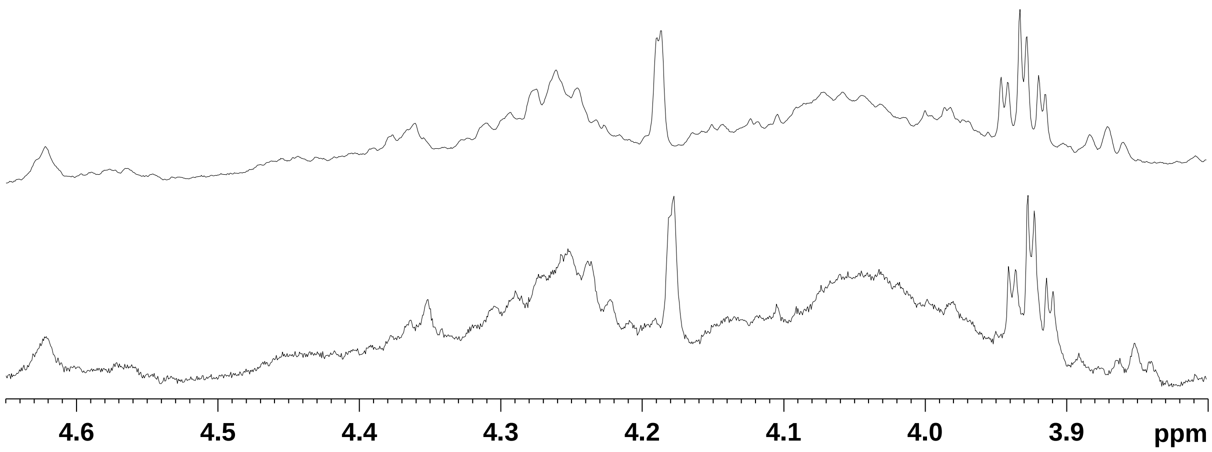

Fig. S2 D_2O shake demonstrating that none of this region corresponds to a hydroxyl proton.

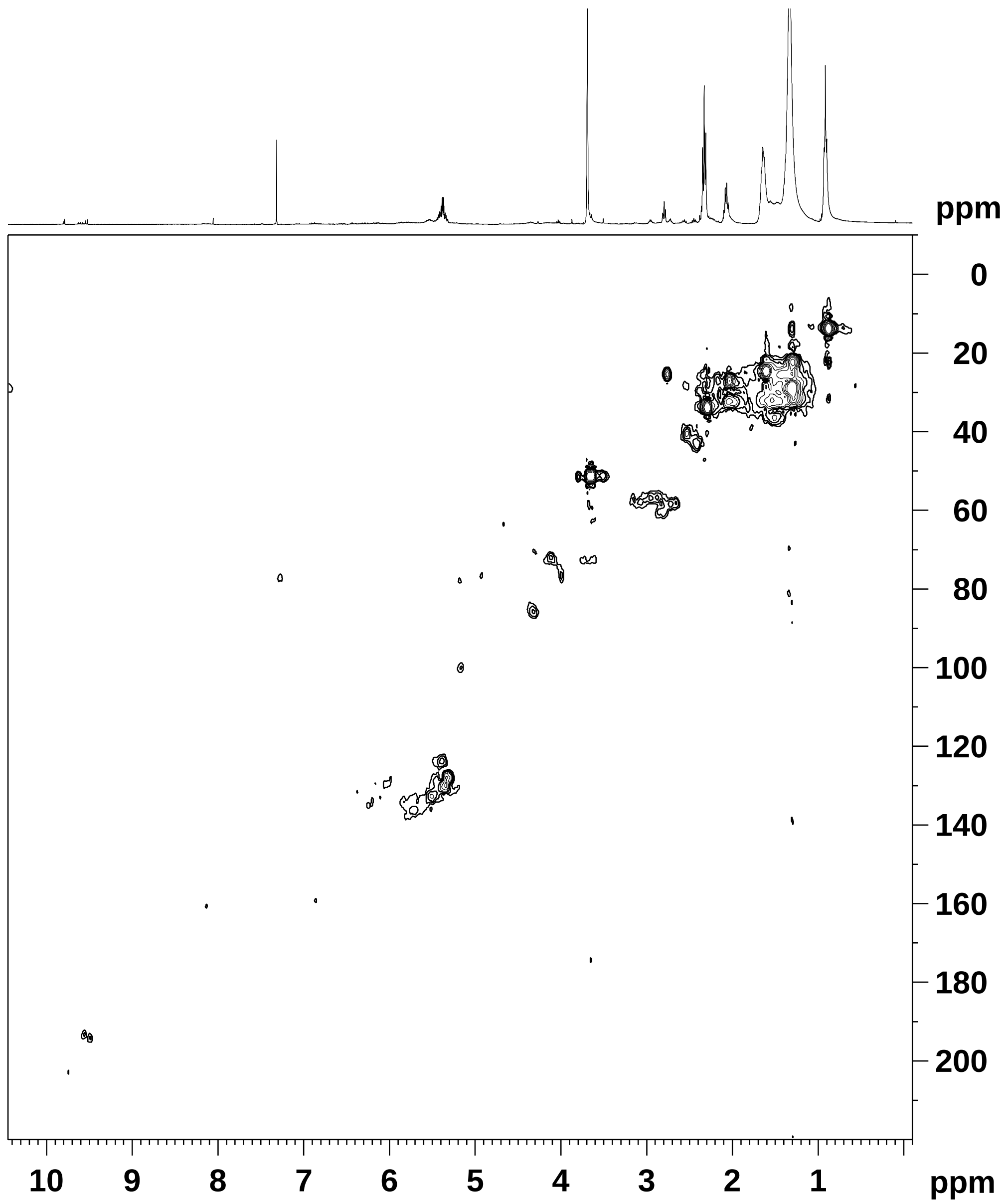

Fig. S3 HSQC PLOT of 18:2 after 500 minutes reaction time

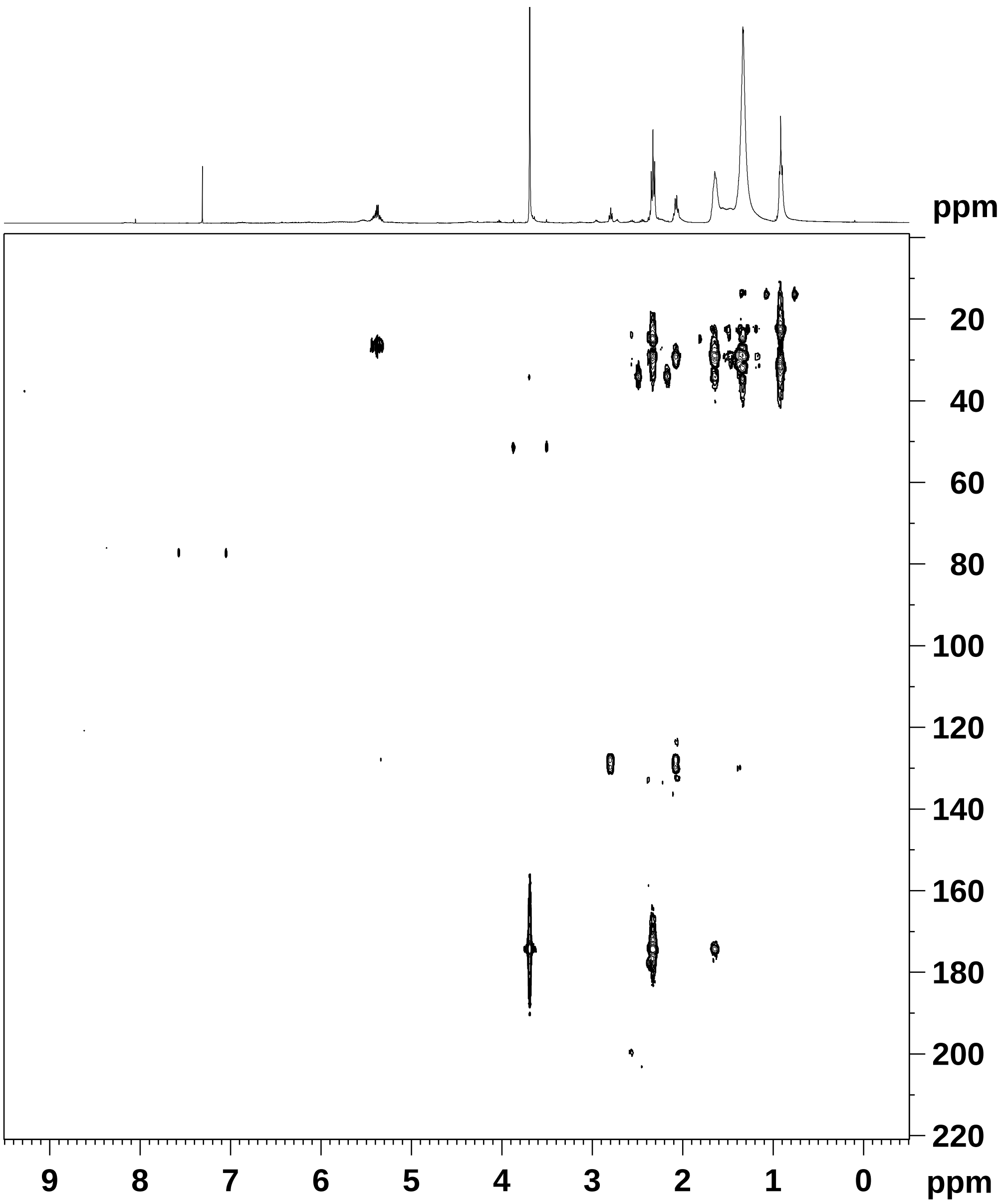

Fig. S4 HMBC PLOT of 18:2 after 500 minutes reaction time