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Catalytic cracking of sterol-rich yeast lipid

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1. Introduction

The mitigation of climate change by reducing global carbon emissions, whilst meeting an ever-increasing

energy demand, is one of the biggest challenges of the 21st century. Emission reductions are particularly

hard to achieve within the transportation sector, which accounts for 26% of total global energy

consumption,[1] mainly due to its heavy reliance on energy-dense liquid fuels. Biofuels are widely seen as an

important contributor to help reduce short- to medium-term emissions until non-carbon based

technologies can be widely introduced. However, for biofuels to have a positive impact on global energy

markets the key technological challenges that need to be addressed are associated with improving the

quality of the fuel produced and ensuring the sustainability of its production.

One of the major sources of biofuels are glyceride lipids, which can be transesterified into biodiesel,

comprised of fatty acid methyl esters. While biodiesel is fully miscible with diesel, fatty acid methyl esters

have high cloud points, low energy densities and low oxidative stability, which have restricted their

maximum blend levels to 7 % (v/v) with ULSD in the EU.[3, 4] Increasing the future use of bio-derived fuels

therefore necessitates the development of higher-quality biofuels with more compatible properties to

USLD. ULSD is a complex mixture of straight-chain and branched alkanes, cycloalkanes and aromatics.

Generally, high levels of long chain alkanes in ULSD will result in higher cetane numbers and higher energy

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density, while the presence of branched components improves the low temperature behaviour. In addition, ULSD typically contains up to 20 wt% of aromatic compounds, which provide vital lubrication for the fuel injection system.

In an attempt to improve the performance of bio-derived fuels, new processes for producing biofuels with similar hydrocarbon compositions to ULSD are being developed and tend to fall within one of two separate approaches.[5] The first approach uses hydrotreating, which involves the deoxygenation of triglycerides into linear chain alkanes, over Pd, Pt, Ni, Ru, Os, Rh or Ir catalysts supported on a range of zeolites, aluminosilicates or activated carbons.[6, 7] This process is followed by additional isomerisation and cracking reactions over Pt or Pd catalysts on zeolite supports, which improve the low temperature properties of the resulting hydroprocessed ester and fatty acid ("HEFA") fuels.[8] A number of commercial hydrotreating processes for producing HEFA fuels have been licenced by companies such as Neste Oil (NExBTL), Syntroleum (Bio-Synfining™) and Honeywell UOP/Eni (Ecofining™) with the first renewable fuel plants having come into operation over the last few years.[9-11] However, as well as consuming significant amounts of hydrogen this method has the drawback of producing fuels with insufficient aromatic content; and must be blended with diesel prior to use to achieve the required lubrication and performance.

The second approach that is capable of converting lipids into suitable fuels with incorporated aromatic components (and the approach that is examined in this work) is catalytic cracking. Catalytic cracking has gained significant interest over the last thirty years after Mobil's development of the ZSM-5 zeolite catalyst. This catalyst was shown to convert a wide range of feedstocks (such as methanol, latex and corn oil) into a uniform product mix with high aromatic content and good selectivity towards a gasoline-type fuel in a single step.[12, 13] Together with ZSM-5, Beta and Y zeolites are the most widely tested zeolites, but have been unable to match the high gasoline yields and low coking performance of the former.[14-16] More recently, attempts have been made to modify the catalyst structure by coating or mixing zeolite catalyst with mesoporous silica, and have helped to increase aromatics selectivities and reduce coke deposition on the catalysts.[17,18] As catalytic cracking is usually carried out at atmospheric pressures of inert

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gas (rather than requiring an external source of H_2, as is the case for hydrotreating) it could potentially result in a comparatively less expensive and more sustainable process for biofuel production. However, this also poses a major challenge, as the lack of hydrogen has been linked to extensive catalyst coking due to the formation of 'allyl-carbenium' ions from the glycerol backbone.[19] Side product formation of hydrocarbon gases, LPGs and solid char is another issue, resulting in low liquid yields of around 40 – 50%.[14, 20-23]

By far the greatest determinant of the sustainability of a biofuel is the sustainability of the feedstock,[24] which has prompted research into alternative sources such as from oleaginous microbes. Many microalgal, fungal and yeast species are classed as oleaginous yet only phototrophic microalgae have been significantly investigated as a biofuel feedstock to date.[25] Microalgae show great potential as a fuel source because of high theoretical lipid yields, but harnessing the CO_2 needed for growth, expensive extraction steps and low growth rates are limiting commercial development. In contrast, oleaginous heterotrophic species, can grow in dense colonies and can produce yields of 100 g L^{-1} over a week in optimized aerobic conditions.[26] While traditionally yeasts are grown on sugars sourced from first generation crops, recent scientific breakthroughs have seen more diverse cellulosic sources (and potentially waste cellulosic materials) being utilised,[27, 28] with certain species producing lipids totalling over 60% of the dry weight under suitable conditions.[29] However, a major challenge in converting microbial lipids is in the complexity of the oils, which can include compounds such as phospholipids, carotenoids and most notably a wide variety of sterols including ergosterol, sitosterol and cholesterol.[30] While sterols are present to up to 5 wt% in algal lipids they can total up to 50 wt% in some yeast oils,[31-34] which can pose problems for processing of these feedstocks via conventional transesterification or hydrotreating routes. For transesterification reactions, the high sterol content would reduce the yield and potentially saponify the catalyst, further increasing the cost of the process. In the hydrotreatment of lipids, contaminants such as alkali metals, phosphorous compounds and the waxes, sterols, tocopherols and carotenoids contained in natural oils and fats can inhibit catalyst performance and may therefore need to be removed prior to processing.[9-11]

1   Despite these concerns, to the knowledge of the authors, no research to date has been published

2 on the effect of converting a sterol-rich microbial lipid into useable hydrocarbon fuels. Here, we

3 investigated the effect of sterol content on the catalytic cracking of rapeseed oil over HY, HZSM-5 and Pd/C

4 catalysts. To determine whether catalytic cracking could be used to provide a hydrocarbon fuel from a

5 sterol-rich lipid feedstock, an unrefined yeast oil derived from the oleaginous yeast *Metschnikowia*

6 *pulcherrima* was also subjected to catalytic cracking and the fuel properties of the resulting liquid product

7 were assessed. The oil from *M. pulcherrima* was selected for this study as the yeast has been previously

8 shown to grow on waste products and under inexpensive non-sterile conditions.[35] This yeast therefore

9 offers a potential economic and sustainable source of lipid.

10

11 **2. Experimental Section**

12 **2.1 Materials**

13 General lab solvents were purchased from Sigma-Aldrich and used without further purification. Deuterated

14 chloroform (CDCl$_3$) for ^{1}H NMR analysis was purchased from Fluorochem. Winter-grade ultra-low sulphur

15 diesel (ULSD-1) with 0 % biodiesel was supplied by the Ford Motor Company. Rapeseed oil was purchased

16 from a local supermarket (Co-operative group vegetable oil made from EU rapeseed) and contained 62 %

17 mono-unsaturates, 30 % polyunsaturates and 8 % saturated esters. Cholesterol (94 %) was purchased from

18 Sigma-Aldrich, UK. Yeast oil was extracted from *M. pulcherrima* according to literature methods.[36] All

19 reagents were de-aerated and dried under standard Schlenk procedures before use. Three industrially

20 relevant catalysts were selected for testing. Palladium on activated charcoal, (5 % Pd basis) was purchased

21 from Sigma-Aldrich, UK and used without further purification. Y zeolite was purchased as ammonium Y

22 zeolite from Sigma-Aldrich with a SiO$_2$ to Al$_2$O$_3$ ratio of 5.02, and calcined at 550 °C for 3 hr to generate the

23 H$^+$ form, according to a procedure specified elsewhere.[37] Pelleted H$^+$-ion exchanged ZSM-5 zeolite (SiO$_2$ to

24 Al$_2$O$_3$ ratio of 38) was purchased from ACS Material, US, crushed, sieved and vacuum oven-dried at 80 °C

4

1   overnight. All catalysts were dried prior to use using standard Schlenk procedures. The crushed HZSM-5

2   and ammonium Y zeolite were both analysed by TGA prior to drying and calcination.

3

4   **2.2 Methods**

5   Conversion reactions were carried out in 50 mL batch reactors (Swagelok sample cylinder 304-L-HDF4-50,

6   sealed with ¼" NPT plugs, SS-4-P, and a high temperature Sealant, MS-TL-SGT). The reactors were loaded

7   under argon with 3 g of reactant and 0.5 g of catalyst. Heating was provided by a tubular pyrotherm

8   furnace with a Eurotherm temperature controller. Reaction temperatures ranged from 300 °C to 400 °C,

9   with a reaction time of 1 or 4 hr, measured from the time the reactor was placed inside the pre-heated

10 furnace. The temperature equilibrium for this set up is rapid, with no more than 5 minutes being needed to

11 bring the reactor to temperature. No pressure monitoring could be performed on the reactors, leading to a

12 potential reaction pressure range from a minimum of 2.0 bar in the absence of any reaction at 300 °C, to a

13 maximum of 40.5 bar at 400 °C for the reaction with the highest gas yield. Following the reaction, the

14 reactors were immediately removed from the furnace and left to cool to room temperature. The

15 chloroform/product solution was filtered prior to chloroform removal by evaporation under vacuum. The

16 filter paper with the solid products was dried in an oven overnight.

17   NMR spectroscopic measurements were carried out at 298 K using a Bruker AV500 spectrometer,

18 operating at 500.13 MHz for ^{1}H. GC-MS analysis was carried out using an Agilent 7890A

19   Gas Chromatograph equipped with a capillary column (60 m × 0.250 mm internal diameter) coated with

20 DB-23 ([50%-cyanpropyl]-methylpolysiloxane) stationary phase (0.25 μm film thickness) and a He mobile

21 phase (flow rate: 1.2 mL min^{-1}) coupled with an Agilent 5975C inert MSD with Triple Axis Detector. A

22 portion of the biodiesel samples (approximately 50 mg) was initially dissolved in 10 mL dioxane and 1 μL of

23 this solution was loaded onto the column, pre-heated to 150°C. This temperature was held for 5 minutes

24 and then heated to 250°C at a rate of 4°C min^{-1} and then held for 2 minutes. The peak area was normalised

25 using the biphenyl internal standard peak area. Each sample was prepared from approximately 100 mg of

product dissolved in 10 mL of dioxane and filtered as necessary. The samples were loaded at 50 °C, and this temperature held for 3 min before heating to 270 °C at 20 °C min^{-1}. Dependent on the sample this temperature was held for between 6 and 20 min. The % areas, used as a semi quantitative method of analysis were calculated from the GC-FID chromatograph. Elemental analysis was carried out by Medac Ltd. The thermogravimetric analysis (TGA) was undertaken on a Seteram TG92 thermogavimetric analyser on ~18 mg samples of yeast solids produced from reaction at 350°C and 400°C. Samples were heated at a rate of 5 $^{\circ}$C min^{-1} in air.

3. Results and Discussion

3.1 Conversion of model oils

To examine the effect of the sterol content on the product distribution, three model biodiesel feedstocks (pure rapeseed oil ("RSO"), pure cholesterol, and a 50:50 mixture of the two ("RC50") were investigated. The catalytic cracking of the three feedstocks was undertaken at temperatures of 300 °C, 350 °C and 400 °C in the presence of a catalyst (either 5 wt% Pd/C, HY Zeolite or HZSM-5 zeolite). 50 mL stainless steel reactors were loaded under argon with 3 g of the chosen feedstock and 0.5 g of catalyst. The reaction time was fixed at 1 hour, as a result of preliminary work demonstrating that 30 minutes was insufficient at lower temperatures and 4 hour reactions yielded very similar product distributions, in accordance with previous studies.[12, 15, 16] The conversions at 300°C were shown to proceed sluggishly, even after an extended reaction period of 4 hours, supporting the use of higher conversion temperatures in industry, which can be in excess of 500 °C. Thus, only the higher temperatures (350°C and 400°C) were used in the following experimental runs.

### 3.2 Mass balances for model compounds

The proportions of the liquid, solid and gaseous product yields (by mass) from cracking reactions on the three model feedstocks at reaction temperatures of 350 °C and 400 °C are shown in Figure 1. For the

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1 production of liquid fuels, the amount of solid and gas produced from the reaction should be minimised,

2 although the deoxygenation of triglycerides will necessarily produce gaseous carbon oxide side products.

3 The solid fraction consists of coke, which is the result of the conversion of the triglyceride backbone in pure

4 vegetable oil,[19] and is an undesirable byproduct that causes fouling and poisons the catalyst surface.

5   The solid fraction of the reaction products was calculated from the weight of the solid residue,

6 minus the initial catalyst weight, following drying in a low-temperature oven overnight. The liquid fraction

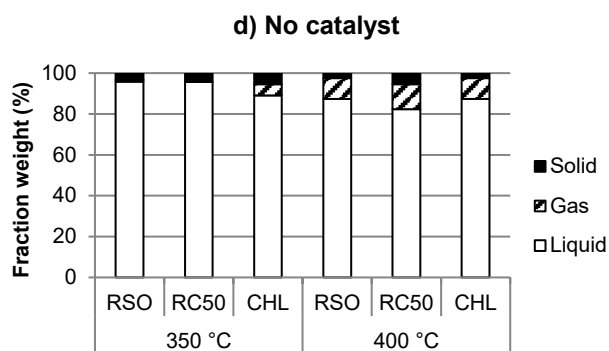7 (defined as the product fraction soluble in chloroform) was recorded as the weight of

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a) Pd/C

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b) HY

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c) HZSM-5

d) No catalyst

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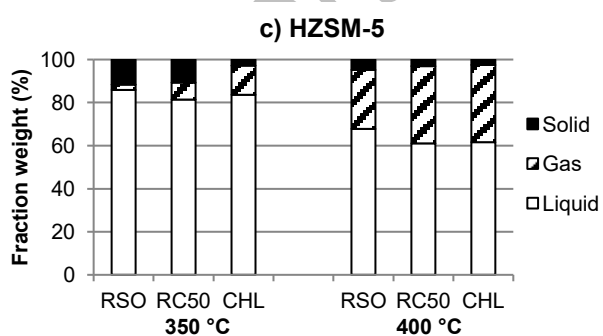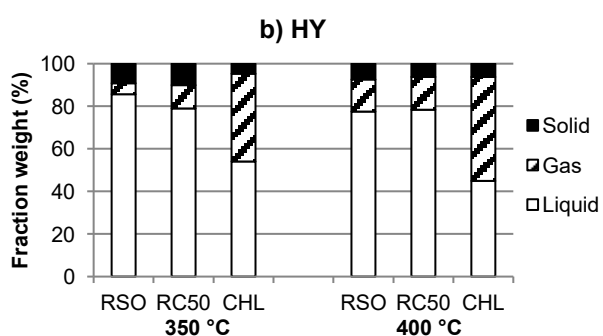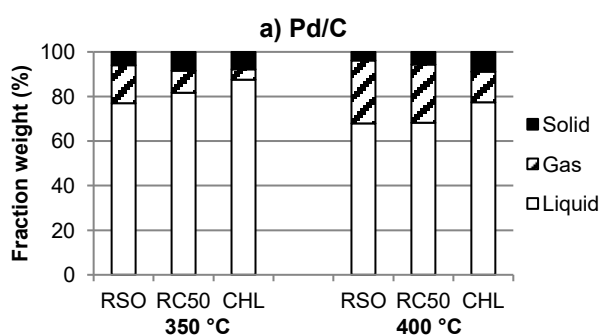
3   *Fig. 1 Proportions of liquid (white fraction), solid (black fraction) and gas-phase (striped fraction)*

4   *products from conversion of rapeseed oil (RSO), RC50 and cholesterol (CHL) at 350°C and 400 °C,*

5   *with no catalyst and in the presence of H-ZSM5, HY and Pd/C catalysts.*

6   the liquid product once the chloroform had been removed via evaporation. The gas fraction (consisting of

7   primarily CO_2, CO and possibly water and short chain alkanes) was calculated from the difference between

8   the weight of the initial feedstock and the combined weight of solid

9   and liquid products. Error bars were plotted based on the standard deviations obtained from three repeat

10   runs for the conversion of RSO over HY Zeolite at 400 °C and Pd/C at 350 °C and the conversion of RC50

11   over Pd/C at 400 °C.

12   In all of the cracking reactions tested here the major product was the liquid fraction (above 75% for

13   RSO at 350°C for all three catalysts). Whilst liquid yields are even higher for the non-catalytic reaction, it

14   must be considered that the successful deoxygenation of the feedstock will necessarily produce gaseous

15   carbon oxides and therefore limit the maximum possible liquid yields. Raising the reaction temperature

16   from 350 °C to 400 °C resulted in an increase in the gaseous component and a drop in liquid product yields

17   of between 10 and 20 wt% over all of the catalysts tested. This is consistent with reports of deoxygeneation

18   of triglycerides in hydrocracking reactions used in industry over Pd, Pt or Ni catalysts, where higher

19   temperatures led to increased cracking activity, increasing the production of gaseous products and

20   consequently reducing liquid yields as well as reducing solid product fractions.[38-40]

For the cracking reactions involving a zeolite catalyst, the introduction of sterol into the feedstock resulted in a further decrease in liquid product yield. Interestingly, the proportion of solid coke produced also decreased with increasing sterol content, with significantly less coking observed in the conversion of pure cholesterol than for the RSO.

In contrast, for the reactions with the Pd/C catalyst, increasing the sterol content resulted in higher liquid yields at both reaction temperatures, perhaps indicating a greater activity for sterol conversion compared to the zeolite catalysts. The proportions of solid coke (4 – 6 %) produced from conversion of RSO over the Pd/C catalyst were lower than for the zeolites at both temperatures (350 °C and 400 °C) and did not have a strong dependence on sterol content.

The solid yields measured for Pd/C were comparable to the relative contribution of the triglyceride backbone to the overall weight of the molecule, of just over 4 % for the RSO feedstock. The higher solid yields obtained over HY and HZSM-5 zeolites decreased in proportion with increased temperature, indicating that they could include reaction intermediates. In contrast, the decomposition of cholesterol and the oil-sterol blends produced a much lower extent of coking with the exception of pure cholesterol on Pd/C. This can be explained by the fact that the decomposition of cholesterol is not hydrogen limited. Due to its multibranched structure, it is expected that extensive cracking of cholesterol would take place over the acid sites in the zeolites, leading to shorter chain alkenes. For Pd/C it is more likely that dehydrogenation of the rings will take place to form multi-ring aromatic compounds. These asphaltene type molecules, common in heavier fuel oil blends, are generally insoluble in common organic solvents such as $CHCl_3$ and as a consequence could potentially result in the apparent increase in solid yields. These results indicated that the lower temperature (350 °C) results in higher liquid yields. In order to investigate the prevalent mechanism for the conversion of sterol-rich feeds, the liquid samples were analysed by ^{1}H NMR.

3.3 Degree of catalytic conversion

1   The degree of triglyceride (TG), double bond (DB) and sterol conversion for the three feedstocks under each

2   set of reaction conditions were calculated from the NMR spectrum relative to the spectrum of the initial

3   feedstock (Figure 2). The degrees of TG and DB conversion are used to indicate the progress of the cracking

4   reaction under these conditions, while DB conversion is also indicative of the oxidative stability of the fuel

5   product (which increases with decreasing DB content). In addition, control experiments were performed to

6   compare the amount of conversion occurring

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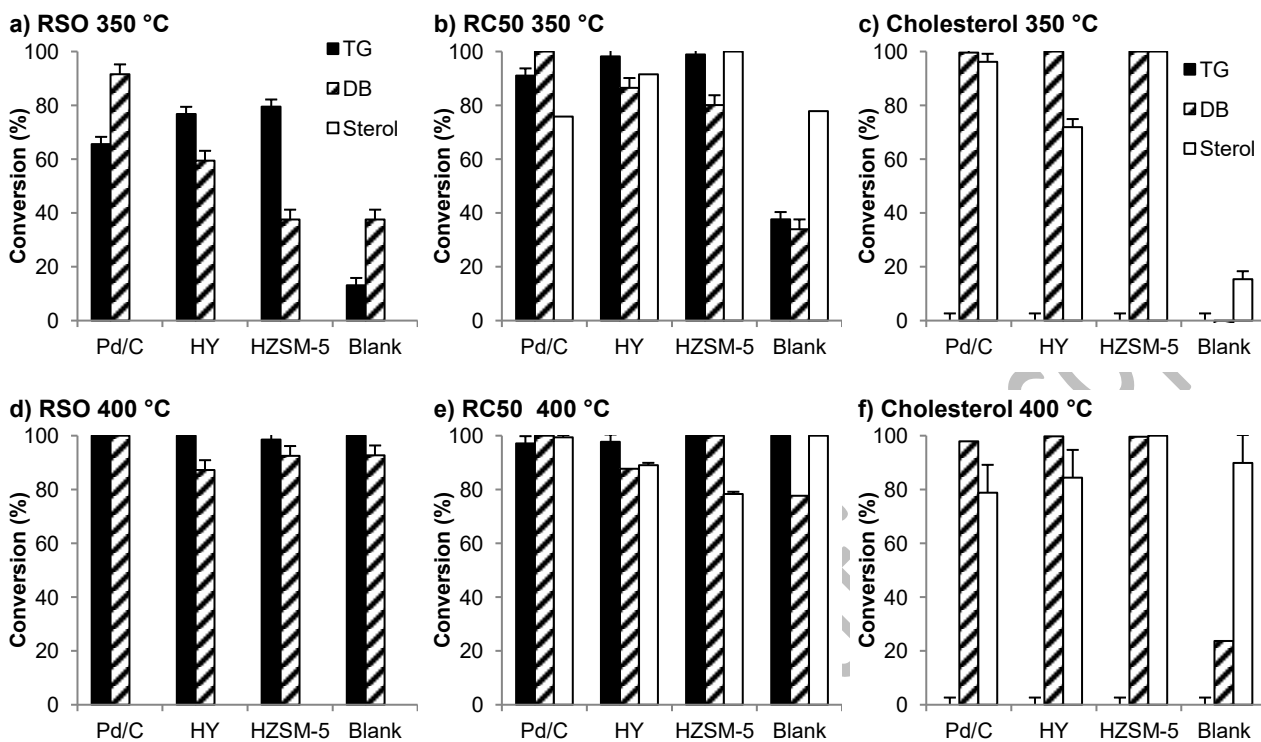
Fig. 2 *Percentage TG (black bar), DB (striped bar) and sterol (white bar) conversions over 1 hour at*

350°C (upper row) and 400 °C (lower row) for RSO (a, d), RC50 (b, e) and 100% cholesterol (c, f), in

the presence of Pd/C, HY and H-ZSM5 catalysts, as well as in the absence of a catalyst (blank).

Percentage conversions calculated from comparison of the integrated intensities from the NMR

spectra

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2   under these conditions in the absence of a catalyst ("blank"). The percentage conversion was determined

3   from the ratios of integrated intensities under the peaks in the NMR spectra of the feedstock and the liquid

4   fuel product (see supporting information for details). The triglyceride conversion was calculated from the

5   percentage difference in integral area corresponding to the protons on the TG backbone ($\delta = 4.0 - 4.5$ ppm)

6   normalised against the integral corresponding to aliphatic protons ($\delta = 0.5 - 3.0$) for the feedstock and the

7   product. Similarly, the DB conversion was calculated from the integral area of the alkene peak ($\delta = 5.0 - 5.5$

8   ppm) minus the area corresponding to the proton on the central carbon of the triglyceride backbone, which

9   overlaps with this region and can be calculated as one quarter of the area corresponding to the C1 and C3

10   protons of the TG backbone ($\delta = 4.0 - 4.5$), normalised against the integral corresponding to aliphatic

11   protons ($\delta = 0.5 - 3.0$). The percentage conversions of the sterol content were calculated from the

12   integrated intensities of the α-proton peaks of the hydroxyl group of the sterol ($\delta = 3.6$ ppm).

13   At the lower temperature of 350 °C (the reaction temperature giving greater liquid yields from the

14   mass balance) it is clear from the conversions in Figure 2 that a catalyst is necessary to achieve TG and DB

15   conversions above 40%. In the absence of a catalyst, low TG and DB conversion levels were observed,

16   presumably catalysed by the steel reactor.

17   The percentage conversion of both the TG and DB components at 350 °C was strongly dependent

18   on the catalyst employed. For the pure RSO just over 60% of the TG in the feedstock was converted over

19   Pd/C, compared with nearly 80% over the zeolite catalysts. However, the Pd/C catalyst appeared to be

20   more efficient at converting double bonds in the RSO at this temperature (~90% compared to <60% for the

21   zeolites). This apparent lack of conversion of double bonds over the zeolite catalysts suggests that

22   unsaturated breakdown products are being formed at the acidic sites. Typically catalytic cracking produces

23   high levels of double bonds, which subsequently oligomerize in the pores of the zeolite to produce

24   aromatic compounds, however, when hydrogen is formed in situ, the double bonds can be hydrogenated

25   yielding mainly alkanes.[41]

1   Interestingly, at 350 °C, for all three catalysts, the percentage TG and DB conversion was higher for

2 the RC50 mixture than for the pure RSO. As seen with the conversion of TG, the zeolite catalysts are more

3 effective at converting the cholesterol than the Pd/C. At 350 °C the DB in the 100% cholesterol feedstock

4 were fully converted by the Pd/C and ZSM-5. The HY zeolite was less efficient at converting the sterol

5 feedstock. This could be due to the smaller number of strong acidic sites in the HY zeolite, compared to the

6 H-ZSM5 catalyst.[42,43]

7   At the higher reaction temperature of 400 °C, virtually all TG was converted irrespective of the

8 sterol in the oil or catalyst identity. Full TG conversion was observed even in the absence of a catalyst. A

9 similar trend was observed with the DB in these feedstocks. The 100% cholesterol feedstock at 400 °C was

10 converted to a similar extent to the lower temperature with HY zeolite being less effective than the other

11 catalysts screened.

12   A less consistent trend was observed regarding the cholesterol conversion over Pd/C. Whilst almost

13 full conversion was obtained at 350 °C, the conversion dropped to less than 80 % at 400 °C, even less than

14 without catalyst. A possible explanation is that the dehydrogenation of the sterol ring adjacent to the OH

15 group stabilises this group and therefore reduces conversion. It must also be considered that the OH group

16 may overlap with similar functional groups on some of the reaction products, leading to a slightly lower

17 apparent conversion.

18   While ^{1}H NMR gives a clear indication of the conversion of the glyceride backbone, or alcohol group

19 of the sterol, it was not possible to determine whether these products had been partially converted into

20 further oxygenated species or cracked into a useable hydrocarbon transport fuel. To further establish the

21 effectiveness of the catalysts, and the products formed, the liquid products were analysed by GC-MS.

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23 **3.4 Product analysis by GC-MS**

24 The GC-MS traces of the liquid products from the cracking of the three feedstocks are provided in the

25 supporting information. A list of the major components recovered is also given in the supporting

13

1   information. Changing the specific catalyst used as well as the temperature of reaction (from 350 °C to 400

2   °C) resulted in significant differences in the product distribution.

3   The liquid fraction obtained from the reaction of RSO with no catalyst at 350 °C and 400 °C

4   contained mainly organic acids with little conversion to hydrocarbon products. The conversion of

5   cholesterol under these conditions yielded numerous fused aromatic rings. The liquid product from the

6   conversion of pure RSO over HZSM-5 and HY at 350 °C also contained predominantly oxygenates, in

7   particular octadecenoic acid and hexadecanoic acid, demonstrating that while the conversion of the TG is

8   almost complete after 1 hr, the deoxygenation step occurs more slowly. The overall contribution of

9   oxygenates was significantly reduced for the products from the RC50 50:50 oil/sterol mixture, as heavier,

10   less volatile compounds were formed. These resembled the multi-ring structure of cholesterol, but could

11   not be clearly identified from the GC-MS data.

12   When the reaction temperature was raised to 400 °C, the conversion of RSO over HY zeolite still

13   yielded significant amounts of octadecenoic and hexadecanoic acid, whilst also producing a range of

14   shorter chain alkanes (C_{10} – C_{15}), the C_{17} and C_{18} compounds undecyl-cyclohexane and dodecyl-benzene and

15   a number of volatile napthalenes. In comparison, the concentration of the direct deoxygenation product

16   from octadecenoic acid, the alkene heptadecene, was low. The relatively poor deoxygenation performance

17   of this catalyst was confirmed by oxygen elemental analysis, which showed a residual oxygen content of

18   7.63% (see supporting information). The addition of 50 % cholesterol led to a reduction of the relative

19   contribution of alkanes, alkenes and acids, and the formation of additional, less volatile compounds. In

20   contrast to HY, the product from the conversion of pure rapeseed oil over HZSM-5 at 400 °C no longer

21   contained detectable amounts of carboxylic acids and only trace quantities of linear chain alkanes or

22   alkenes. Instead, its main components were short-chain single-ring aromatics such as 1-ethyl-2-methyl-

23   benzene and the C18 aromatic dodecyl-benzene. ZSM-5 catalysts are generally found to be more selective

24   towards aromatics than Y zeolites,[14-16] with the differences attributed to the different accessible pore sizes

25   of the various zeolites. Whilst the ZSM-5 pores (consisting of 10-membered rings) are large enough to

14

1 accommodate a single benzene molecule and thereby favour the formation of aromatics, the larger pores

2 in Y zeolites (12-ring) may allow multiple molecule condensation, resulting in the increased formation of

3 coking products.[16] For the ZSM-5 catalysed reaction, the addition of sterol had a lesser impact on the

4 product distribution than for HY, with the exception of a reduction in the dodecyl-benzene yield and the

5 formation of the less volatile sterol decomposition products.

6   In contrast to the zeolites, the conversion of TG over Pd/C gave a much narrower product

7 distribution, showing a high concentration of the straight chain alkanes pentadecane and heptadecane at

8 both conversion temperatures. The reaction also produced high concentrations in the single ring C17

9 aromatics 1-methyldecylbenzene and undecylbenzene, whereas few oxygenated compounds could be

10 detected. Again these findings could be verified by oxygen elemental analysis which showed an oxygen

11 content of 6.89 % for the conversion of RO at 350 °C and only 1.40 % for the RC50 mixture at 400 °C (shown

12 in supporting information). For all the catalysts tested, higher temperatures led to improvement in the

13 selectivity towards alkanes rather than oxygenates and increased C_{17}/C_{18} ratios.

14   Based on the GC-MS results, the reactions of RSO and 100% cholesterol over Pd/C and the two

15 zeolites appear to follow very different mechanisms. Whilst Pd/C results in high levels of deoxygenation at

16 350 °C, the higher temperature of 400 °C is required to achieve significant deoxygenation over the two

17 zeolite catalysts. As the deoxygenation products from Pd/C are predominantly C_{15} and C_{17} hydrocarbons, it

18 can be concluded that deoxygenation proceeds either via decarboxylation or decarbonylation. In the case

19 of the two zeolites, the presence of both C_{17} and C_{18} compounds suggests that deoxygenation also follows

20 the hydrodeoxygenation pathway. Whilst no water was detected in the reaction product, this may have

21 been lost during the extraction process or reacted further with the hydrocarbon products to produce CO_2

22 and additional hydrogen to drive the reaction.

23   Nevertheless the hydrodeoxygenation reaction requires significant amounts of hydrogen which,

24 under inert conditions, must be produced in situ by dehydrogenation or aromatisation of the fatty acid

15

1 chains. Zeolites are poor hydrogenation catalysts and the low concentrations of alkenes relative to

2 aromatics suggest that aromatisation is the preferred mechanism.

3 Using the Pd/C catalyst, the main reaction products from the conversion of rapeseed oil were

4 pentadecane and heptadecane from the decarboxylation of the C_{16} and C_{18} esters, and the aromatics

5 methyldecylbenzene, undecylbenzene and 1,3-dimethylbutylbenzene, presumably derived from the

6 unsaturated esters. The catalytic cracking of cholesterol at 350 °C over Pd/C yielded a range of heavier

7 aromatic compounds, such as phenyl-substituted naphthalene. The product distribution is relatively small

8 with less than 6 major components. An overabundance of these multi-ring compounds would be

9 detrimental to the low temperature properties of the resultant fuel as they form waxy solids which increase

10 the cloud point and viscosity. The heavier components seen at 25 minutes in this trace, seemingly

11 oxygenated multi-ring compounds, were not observed at 400 °C, indicating that under these conditions the

12 ring structure has been successfully cracked into a range of short chain alkanes and lighter, mono-aromatic

13 species. The addition of 50 % cholesterol to rapeseed oil resulted in the formation of many of these

14 components, such as hexaethyl benzene, but also included heavier, multi-ring compounds. In addition, it

15 led to the formation of low amounts of heneicosane (C_{21}) and tetracosane (C_{24}), but more significantly,

16 resulted in an increase in the alkane to aromatics ratio at both temperatures. This increase in the

17 proportion of alkanes suggests that cholesterol may donate hydrogen to the reaction and thereby inhibit

18 the formation of aromatics from the triglycerides. In this case hydrogen is released through the

19 aromatization of the cholesterol rings over Pd/C, as evidenced by the formation of aromatized cholesterol

20 derivatives as shown in the supporting information. It also corresponds with previous studies in the

21 literature that suggest that the aromatic content of the product is dependent on the number of double

22 bonds in the feed oil.[19]

23 It is clear in the conversion of sterol-rich lipids that while the zeolites gave carboxylic acid products

24 and large amounts of aromatics, cracking over Pd/C gave a more diesel-like composition of low oxygenates,

1   straight chain alkanes and acceptable levels of aromatics. This catalyst was therefore selected for use in the

2   following section to convert an unrefined sterol-rich microbial oil into a hydrocarbon fuel.

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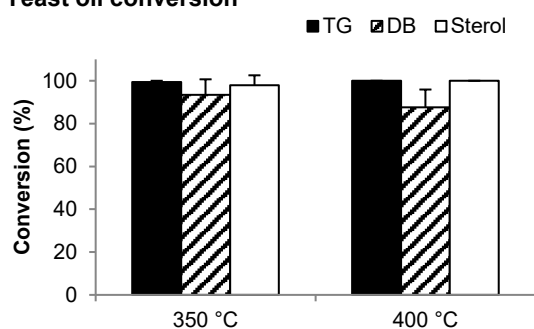4   **3.5 Conversion of yeast oil**

5   The yeast oil selected for this study was extracted from the oleaginous yeast *Metschnikowia pulcherrima*,

6   and contained 18 mol% sterols. The elemental analysis of the oil showed elevated levels of both oxygen

7   and phosphorous compared to rapeseed oil, indicating the presence of small chain oxygenates and

8   phospholipids. A full breakdown of the elemental analysis and lipid profile is given in tables S1 & S2 in the

9   supplementary information. Catalytic conversions of yeast oil were performed at 350 °C and 400 °C. The

10   analysis of the product proportions and percentage conversions after 1 hr reaction over a Pd/C catalyst are

11   presented in Figure 3a and 3b. The mass balances performed on the products of the cracking reactions

12   showed that the conversion of the yeast oil produced a much higher proportion of solids, of up to 22 %,

13   than the reaction of RSO and the RC50 oil/sterol mix. The thermogravimetric analysis (TGA) of the solid

14   product formed at 350 °C indicated that the majority of the solids (~60%) was char, where roughly 40% was

15   organic asphaltene compounds typically found in heavier fuel oils (see supporting information). At 400 °C,

16   where 17 wt% solids were recovered, less than 1% of the solids were found to be organic molecules and

17   the remainder of the solid product was comprised of coke. This higher level of coking is presumably the

18   result of the presence of phospholipids, organic acids and metal contaminants as suggested by the

19   elemental analysis of the oil. Unlike rapeseed oil, which is refined during the production process, the yeast

20   oil was not purified following extraction. Consequently liquid yields were significantly lower. Gas yields in

21   turn were similar at 400 °C, whereas at 350 °C a slightly higher proportion of gas was formed from the

22   reaction of yeast oil than that of the model compounds. What is encouraging to note is that under

23   conditions of 350 °C and 1 hr, a liquid product yield of 60% could be achieved over a Pd/C catalyst. The TG,

24   DB and sterol conversions demonstrate that the triglycerides in the yeast oil are much more easily

25   converted than those in RSO, as full TG conversion is achieved at 350 °C, compared to only 66 % and 88 %

17

a) Yeast oil mass balance
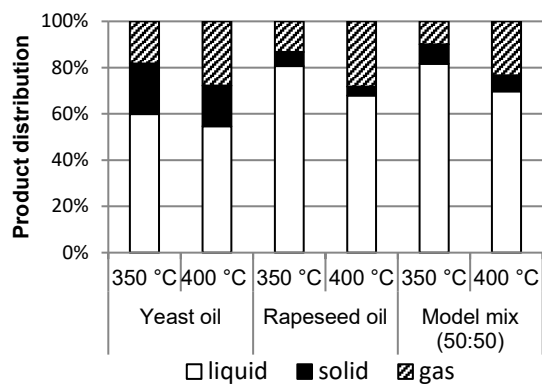
b) Yeast oil conversion

Fig. 3 *a) Proportions of liquid, solid and gas-phase products from conversion of yeast oil at 350°C and 400 °C*

(1 hr), in the presence of a Pd/C catalyst. The product distributions from the RSO and RC50 model

compounds under the same conditions are shown here for comparison; b) Percentage TG, DB and sterol

conversions, calculated from NMR spectra, over 1 hour at 350°C (left) and 400 °C (right), for yeast oil in the

presence of a Pd/C catalyst.

1   for pure RSO and the RC50 mixture, respectively, under the same conditions. The components of this liquid

2   fuel were consequently examined via GC-MS (Fig. 4).

3   The conversion of the yeast oils produced a higher relative proportion of pentadecane than the

4   conversion of rapeseed oil as a result of the greater proportion of C_{16} esters in the feedstock oil. The major

5   products formed at both temperatures were pentadecane and heptadecane. The products also contained

6   significant amounts of hexadecane and octadecane, the shorter chain alkanes undecane through to

7   tetradecane, as well as the longer chain alkanes nonadecane and tetracosane. At 350 °C, approximately 3 %

8   of the hexadecanoic acid was observed, whereas full conversion was achieved at 400 °C. In contrast, no

9   residual amounts of octadecanoic acid were detected at either conversion temperature although small

10 quantities of a C_{18} oxalic acid derivative were present. These could be a reaction product of octadecanoic

11 acid with oxalic acid impurities in the yeast oil. The reaction products contained significant amounts of the

12 alkenes hexadecene and heptadecene and as already indicated by the NMR results, the alkene content is

13 higher for the products from the reactions at 400 °C. Oxygen elemental analysis showed that a high degree

14 of deoxygenation was achieved at both temperatures, resulting in residual oxygen contents of 4.06 % at

15 350 °C and 2.49 % at 400 °C (see supporting information).

16   The products also contained a range of C_{17} and C_{18} single ring alkanes and aromatics, such as 1

17 methyldecylbenzene and cyclopentadecane, and the presence of undecylcyclohexane and undecylbenzene,

18 as well as dodecylcyclohexane and dodecylbenzene. This suggests that the aromatics are formed via

19 cyclohexane ring intermediates. It has previously been proposed that the formation of aromatics is strongly

20 related to the number of double bonds in the feedstock,[19] so the lack of C_{16} polyunsaturates in the yeast oil

21 could explain the absence of C_{15} or C_{16} chain aromatics in the GC-MS. The location of the six-membered ring

22 at the end of the of the alkyl chain suggests that the aromatics are formed by the reaction of the terminal

23 end with the double bond present in the 18:2 and 18:3 fatty acids. This also explains the high quantity of 1-

24 methyldecylbenzene which results if the bond is formed between the terminal end and the C_7 carbon, and

25 subsequently re-arranged to form the more stable six-membered ring structure.

19
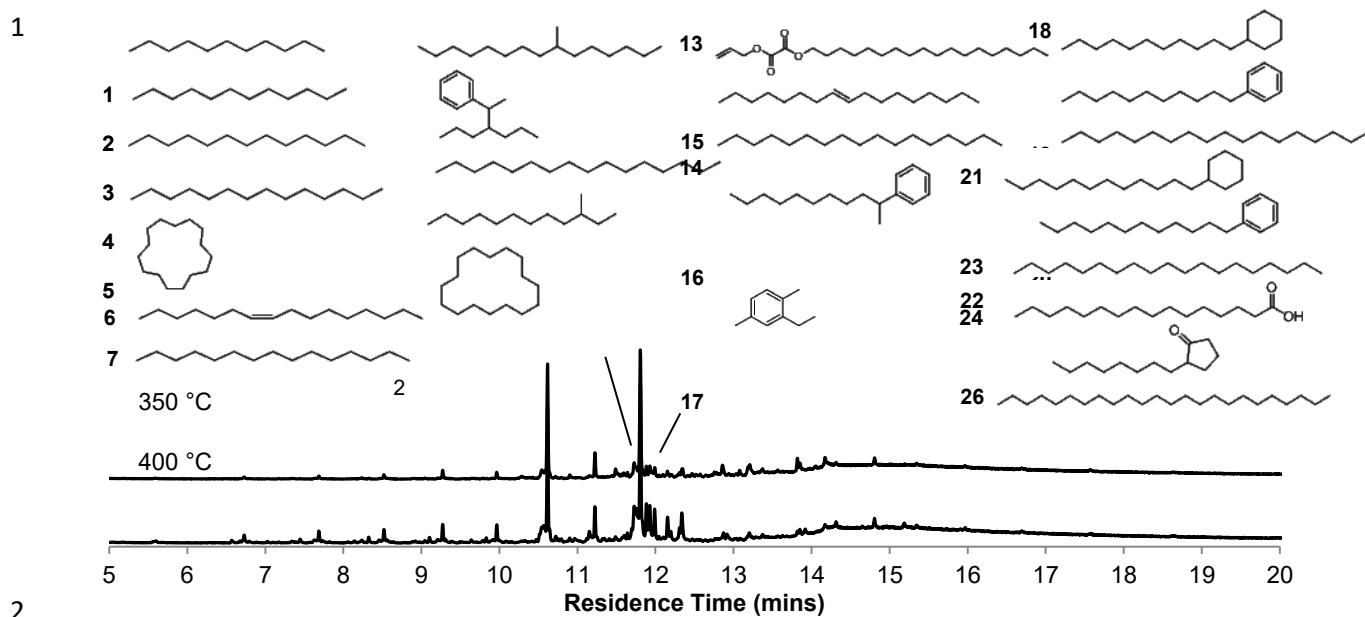
Fig. 4 GC-MS chromatograph of the conversion of yeast lipid at 350 and 400 °C with the major peaks

annotated, all products are observed in both traces except compounds 5 and 9, only observed at 400

1 The alkane to aromatics ratios, calculated by dividing the sum of the percentage signals corresponding to

2 alkanes by the sum of percentage signals corresponding to aromatics, were higher at 350 °C (approximately

3 2.8 to 3.0) than at 400 °C (approximately 1.8 to 2.0) and significantly higher than the ratios obtained from

4 the conversion of pure rapeseed oil (approximately 0.9 (400°C) to 1.0 (300 °C)) but lower than those for

5 the RC50 mixture (approximately 3.2 (400°C) to 3.7 (350°C)). This suggests that the sterols present in the

6 yeast oil are acting as a potential source of hydrogen and result in increased formation of alkanes. As the

7 amount of sterol in the yeast oil is lower than in the RC50 model mix (approximately 9 wt% c.f. 50 wt%), a

8 lower alkane to aromatics ratio is obtained.

9   The presence of small quantities of octadecane and hexadecane suggest that the deoxygenation

10 pathway is partially shifted towards hydrodeoxygenation. Again, the required hydrogen could be produced

11 in situ by the dehydrogenation of the fatty acid chains, as shown by the presence of hexadecene in the

12 reaction product. The relative higher concentration of hexadecane to octadecane indicates that the

13 hydrodeoxygenation mechanism is facilitated for more saturated alkanes. In contrast, the

14 hydrodeoxygenation mechanism is inhibited by the high number of double bonds in rapeseed oil, and

15 therefore no C_{18} products were formed during its conversion. As the GC-MS demonstrated that cracking of

16 the yeast oil resulted in a complex mix of compounds including the desired aromatics, the fuel properties of

17 the resulting fuels were then examined and compared to ULSD.

18 **3.6 Fuel properties**

19 The deoxygenation of yeast oil over Pd/C produced a high content of pentadecane, heptadecane and long

20 chain single ring aromatics. Pentadecane and heptadecane both display very high cetane numbers (96 and

21 105 respectively) where the long chain aromatics have cetane numbers between 51-68. This is indicative of

22 a highly promising blend for diesel fuel, which requires minimum cetane numbers of 51 in the EU.[4, 44, 45] The

23 fuel properties of the hydrocarbon fuels were analysed at various blend levels with mineral diesel (denoted

24 here as USLD-1) and compared to the European diesel fuel standard EN 590. While there is no maximum

25 cloud point requirement for temperate climatic zones, a cloud point of -10 °C or lower is required for most

21

1   European countries between 16th November and 15th March.[4] This is achieved though further refining and

2   additional cloud point suppressant additive packs.[46] The cloud points above freezing were determined for

3   the fuel product using a combination of water and ice baths. Cloud points below freezing were determined

4   using a Dairei DP-80 Cryo Porter freezer. The cloud point of the pure yeast derived fuels is reasonably high

5   and more akin to biodiesel than mineral diesel (Fig. 5a). The blending with diesel resulted in an almost

6   linear decrease in cloud points for all products. The cloud point of the fuels approached -10 °C at a diesel

7   concentration of 50 %, though winter fuel additives would be needed beyond this blend level to bring the

8   fuel into the required diesel range.

9   Gravimetric densities of the two yeast oil fuels were measured at 40 °C by weighing 1 mL samples

10   of product. The acceptable fuel density range for ULSD at 15 °C is specified as 820 to 845 kg m^{-3}. Both of the

11   pure yeast fuel products have higher density than the upper limit set out in EN 590 (Fig. 5b). This in contrast

12   to the fuels produced from hydrotreating and isomerisation, which tend to have densities below the

13   acceptable range. This difference can be attributed to the high relative aromatic and straight chain alkane

14   content of the yeast derived fuels.[31,32] The addition of ULSD in turn results in a roughly linear decrease in

15   fuel density, with both fuel products falling within the acceptable range at a 25% blend level. It is unlikely

16   that these slightly higher densities are a major issue. Fossil diesel has a relatively constant energy density,

17   and the gravimetric density is an important factor to regulate the maximum power output and maximum

18   volumetric fuel consumption.[32] As a result, low fuel densities may result in decreased engine performance.

19   In contrast, the high densities seen for the deoxygenation products are expected to result in improved

20   engine performance. The potential improvement that a high density bestows is also reflected in the energy

21   density (Fig. 5c). In order to obtain an energy density, the energy content of the neat products was

22   measured in a Parr 1341 Plain Oxygen Bomb Calorimeter, using the method set out in ASTM D240. As a

23   function of mass the fuel produced at 350 °C had an energy density that is 4% higher than the ULSD-1 diesel

24   used in this study. The fuel produced at 400 °C had the same energy density by mass as ULSD-1. However,

25   due to the increased density the volumetric energy density was 14% higher for the fuel produced at 350 °C

22
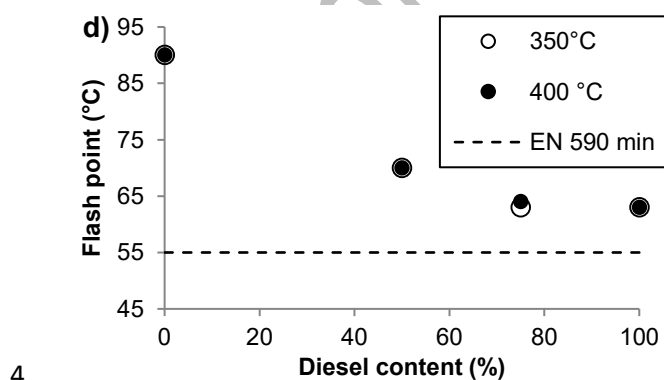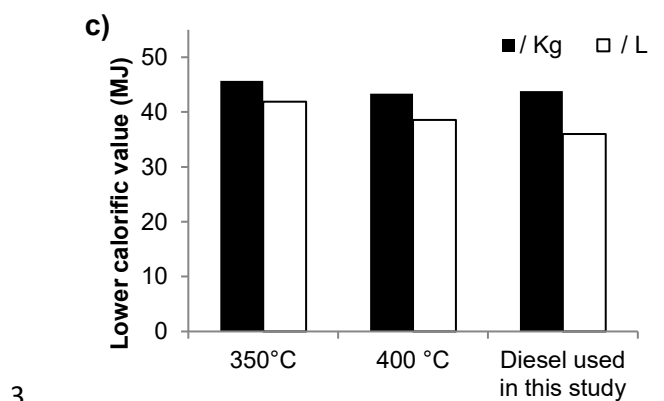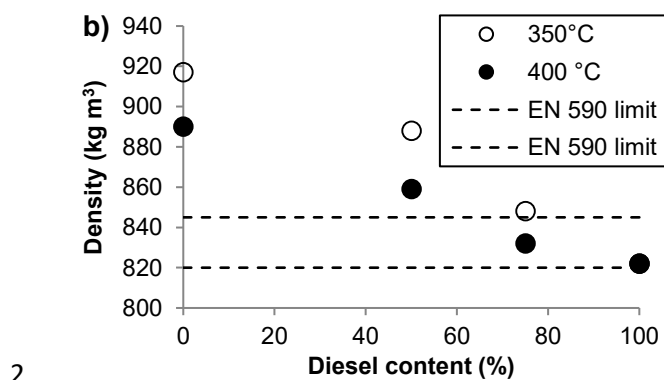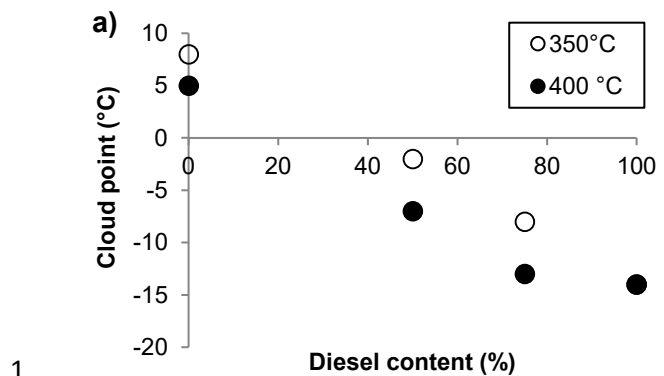
Fig.5 *Fuel properties of the pure and diesel blends of the two yeast derived hydrocarbon fuels produced at 350 °C and 400 °C. The cloud point is given in (a), the density in (b), the energy density (lower calorific value) in (c) and the flash point (d).*
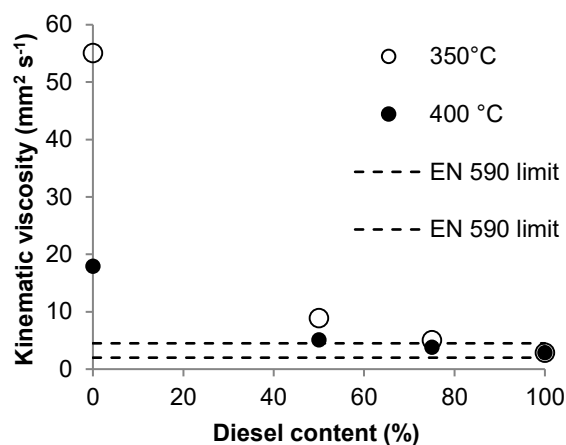
23

Kinematic viscosity (mm² s⁻¹)

○   350°C
●   400 °C
- - - - EN 590 limit
- - - - EN 590 limit

0   20   40   60   80   100
Diesel content (%)

1
2   **Fig. 6** The kinematic viscosity of the pure and blended yeast derived fuels

3   and 7% higher for the fuel produced at 400 °C. This would mean that a vehicle driven with a blend of yeast

4   derived fuel would have a better fuel mileage than one driven with only ULSD-1.

5   The European Standard specifies a minimum diesel flash point of 55 °C. Flash Point testing was

6   conducted using a Stanhope-SETA 99880-0 Flashcheck Instrument using the TAG closed cup method set out

7   in ASTM D56. The flash points for both fuels and their diesel blend levels comfortably exceed this limit. This

8   is not surprising, as the conversion products only contain a small percentage of volatile compounds which

9   are able to form a combustible vapour phase.

10   The acceptable range of kinematic viscosity at 40 °C is 2.00 mm² s⁻¹ to 4.50 mm² s⁻¹. The kinematic

11   viscosities of the product/diesel blends were measured at 40 °C with a calibrated Canon-Fenske Routine

12   Viscometer No. 200 according the method set out in ASTM D445. Whilst the viscosities of the products

13   blended with diesel were measured using a calibrated viscometer as specified in standard EN ISO

14   3104:1996, the viscosity was too high for the hydrocarbon fuels produced from the pure yeast oils to

15   determine this directly. Instead, the dynamic viscosities were measured using a Bohlin rheometer and

16   converted to kinematic viscosities by dividing the dynamic value with the density of the sample. The

17   dynamic viscosities of the neat products were measured on a C-VOR 200 Bohling Rheometer (cone and

18   plate: 20 mm, 1 °) at 500 rpm. The viscosities of the pure reaction products exceed the maximum value by a

24

1 large margin (Fig. 6). This is presumably due to the lack of short chain alkanes and isomerised species in the

2 fuels, resulting in strong intermolecular attractions, as already demonstrated by their high cloud points.

3 The viscosities could be significantly reduced by blending with diesel, with the blends of products

4 only slightly exceeding the upper limit of 4.50 mm^2 s^{-1} at 50:50 blends and meeting the required viscosities

5 at 25:75 blends. Again the fuel produced at 350 °C has a higher viscosity than that at 400 °C, as can be seen

6 by the larger proportion of shorter chain alkanes, and lower acid content, in the GC-MS chromatograph of

7 the 400 °C product. The high viscosity of the fuel blends precludes the use of this yeast oil in its pure form

8 and rather it is only in a blend that it would be suitable for road transport use. Further to road use, marine

9 diesel is another suitable application that requires aromatics for lubricity. A far higher viscosity is

10 acceptable (up to 11 mm^2 s^{-1}) is allowable for marine distillate fuels and up to 700 mm^2 s^{-1} for marine

11 residual fuel oils.[47] The reaction products from the yeast oil could potentially be used unblended for these

12 types of application.

13

14 **4. Conclusion**

15 Lipids derived from heterotrophic organisms have the potential to become a fundamental feedstock for

16 future transport fuels, however, the effect of their high sterol content on their conversion had not been

17 thoroughly investigated. In this investigation we have shown that not only can sterols be converted by

18 catalytic cracking using relatively short reaction times, but by using a Pd/C catalyst, a fuel with a high

19 energy density, high flash point and high proportion of high cetane number compounds as well as a

20 reasonable level of aromaticity can be produced from an unrefined sterol-rich microbial oil. This conversion

21 can be done without the addition of H$_2$ in a single step making this a potentially more sustainable fuel.

22 In addition, the conversion of rapeseed oil and cholesterol models were tested at catalytic cracking

23 conditions over the three catalysts Pd/C, HY Zeolite and HZSM-5 zeolite. Full triglyceride conversion was

24 achieved over all three catalysts, with slightly higher activities over the two zeolites. In contrast, Pd/C

25 proved a significantly more active deoxygenation catalyst, with high levels of deoxygenation achieved at

25

the reaction temperature of 350 °C, compared to 400 °C for the zeolites. The addition of cholesterol to rapeseed oil resulted in a significant reduction in solid coke, especially for the two zeolites, and led to the formation of less volatile, multi-ring compounds, though a greater proportion of these heavy compounds could be successfully cracked using the higher reaction temperature of 400 °C. Cholesterol addition also led to a significant increase in the alkane to aromatics ratio obtained over Pd/C, which showed that it could potentially be used as a hydrogen donor to influence the aromatic content of the product.

The Pd/C catalyst was highly active in converting yeast lipid, containing 18 mol% sterol, derived from *M. pulcherrima*. Conversion rates were comparable to those of rapeseed oil, with a significant increase in the relative contribution of hydrodeoxygenation to the overall deoxygenation reaction. This increase was attributed to a higher degree of saturation in the yeast oil. The key fuel properties of the liquid products were tested and compared to the specifications set out in BS EN ISO 590:2009. Although none of the pure products met the requirements, especially due to their high viscosities and cloud points, at 50 % blends with diesel almost all products fell within the diesel specifications. This work has also demonstrated that blending yeast oil-derived fuel with ULSD in excess of the maximum 7% v/v biodiesel blend stipulated by the EU can result in a fuel with potentially higher performance than unblended ULSD.

Acknowledgements

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