Triglycerides, the main components of plant and animal lipids, are becoming increasingly important as a feedstock for a number of industrial applications. Aside from the food industry, the two dominant uses of glyceride lipids are in the formulation of care products and in the production of biodiesel. The fuel properties, and therefore suitability of biodiesel, are dependent on the lipid profile. Biodiesel, comprised of mainly saturated esters such as palm oil methyl ester, has both a high cloud point and viscosity. This can lead to increased particulate emissions and filter blocking particularly at low temperatures [1]. By contrast, biodiesel comprised of mainly polyunsaturated esters has vastly improved low temperature properties, but has a poor cetane number and low oxidative stability [2]. Consequently, the biodiesel standard EN 14214 mitigates against these poor properties by limiting the amount of polyunsaturated esters by limiting the iodine value, and specifically caps the amount of linolenic acid ester in the fuel. Similarly, the kinematic viscosity of the fuel is limited to a maximum of 5.0 mm²s⁻¹ at 40°C, which effectively restricts the amount of saturated esters present [3]. Rapeseed methyl ester, predominantly oleic acid methyl ester, has a pour point of around -9°C, depending on the source crop, whereas palm oil methyl ester can have a pour point of 16°C or higher [2]. Therefore, biodiesel comprised mainly of oleic acid alkyl esters has properties more akin to mineral diesel and, therefore, has a higher performance in compression ignition engines [4].

Another major use of lipid oils is in the care product industry, for surfactants, and in foodstuffs where a high viscosity and high melting point are generally desirable [5]. The lipid source of choice in these industries is palm oil [6,7]. Approximately 85% of all palm oil is produced in Indonesia and Malaysia, where an accelerating demand is contributing to a 1.5% annual rate of deforestation of tropical rainforests [8]. In addition, catastrophic localized ecological damage such as land-use change are associated with large GHG emissions. Fargione et al. estimated that converting a hectare of rainforest to palm oil cultivation produced 1294 Mg CO₂, and this carbon debt would take 423 years of biofuel production to pay back [9]. The same report notes that the production of fuels from lignocellulosic wastes attracts no such penalty. A key sustainability goal is therefore to replace lipids necessary

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Background: Lipids are an increasingly important chemical feedstock for the manufacture of biofuels, bioplastics, care products and as a food source. Developing sustainable sources of lipids, derived from oleaginous microbes, is therefore a key scientific challenge. Methodology: Design of Experiments was used to optimize the lipid production and lipid profile. Results: Here we successfully apply Design of Experiments to optimize the lipid profile in Rhodotorula glutinis to tailor the fatty acid profile. A high culture temperature and high nitrogen ratio yielded a mainly monounsaturated oil, while low temperatures and high glucose loadings gave a more saturated profile. Conclusions: On transesterification, the oil high in monounsaturated esters yielded biodiesel with fuel properties akin to rapeseed methyl ester, whereas the oil high in saturates was found to be suitable as a substitute for palm oil.

Optimizing the lipid profile, to produce either a palm oil or biodiesel substitute, by manipulation of the culture conditions for Rhodotorula glutinis

Biofuels (2014) 5(1), 33–43
to the fuel and care product industries with renewable alternatives sourced from lignocellulose.

One potential alternative is to cultivate oleaginous microbes heterotrophically on lignocellulose feedstocks sourced from waste biomass, or from biomass grown on degraded and abandoned agricultural lands. Some yeasts, such as select species from the genera *Yarrowia*, *Candida*, *Rhodotorula*, *Rhodosporidium*, *Cryptococcus*, *Trichosporon* and *Lipomyces*, are capable of accumulating intracellular lipids up to 80% of their dry biomass weight [10]. However, of the more than 600 yeast species known, fewer than 30 are known to accumulate more than 20% of their biomass as oil [11]. One of the most promising yeasts for lipid production is *Rhodotorula glutinis*, which accumulates up to 60% dry weight in lipids using glucose as the carbon source [12]. *R. glutinis* grows on a variety of carbon sources including hexose and pentose sugars [13], as well as waste streams such as crude glycerol [14], thin stillage [15] and waste whey [16]. Biomass production can be as high as 185 g/l when grown in a fed-batch culture [17]. Typically, the lipid profile obtained from *R. glutinis* contains palmitic acid (16:0), oleic acid (18:1) and linoleic acid (18:2) as the main lipids. *R. glutinis* has also attracted research attention due to its ability to produce carotenoids, lipid-soluble pigments, the majority of which are C<sub>40</sub> terpenoids [16]. These compounds are of commercial importance as food colorants, nutritional supplements and natural antioxidants. To provide the correct physical properties for use, any replacement oil requires a fatty acid profile similar to the oil that it is displacing. While metabolic engineering of microbes is one method of tailoring the lipid profile to desirable products [18], microbes also produce different lipids depending on the environmental conditions [19]. By changing the carbon-to-sulfur of the growth medium, Wu *et al.* demonstrated that the fatty acid composition of *R. toruloides* can be tailored accordingly; for example, a higher cultural carbon-to-sulfur molar ratio favored saturated fatty acids [20]. Similarly, *Yarrowia lipolytica*, a yeast that produces a single-celled oil rich in 18:0, could be used as a substitute for cocoa butter when co-cultured in the presence of an oleic acid donor, such as hydrolyzed rapeseed oil [21]. A number of other strategies have also been investigated to increase the level of saturated lipids in yeasts, to produce an effective replacement for cocoa butter or palm oil [21]. These include crystallizing out the saturated fats at low temperatures, including Δ9 and Δ12 desaturase inhibitors in the culture media [22], genetic manipulation [23,24] and O₂ depletion [25]. These techniques have been applied to a range of yeasts strains from the species *Lipomyces starkeyi*, *R. toruloides*, *Apiotrichum curvatum*, *Candida curvata*, *Cryptococcus curvatus*, *Trichosporon fermentans* and *Y. lipolytica* [26]. Arguably, however, the simplest method to increase saturated esters is to manipulate the temperature or nitrogen content of the culture. For example, the amount of saturated esters was increased by 10% on reducing the culture temperature for *C. curvatus* [26], while the production of polyunsaturated esters could be reduced substantially on culturing various Zygomycetes at lower temperatures [27].

Statistical experimental design techniques, especially the response surface methodology (RSM), are extremely useful in understanding the effects and interactions of multiple factors. Medium optimization for lipid production using RSM has been used previously for oleaginous microorganisms including *R. glutinis*, grown on crude glycerol [14], and the co-fermentation of glucose and xyllose using *L. starkeyi* [28]. Surprisingly, given the potential of the yeast, the effect of the culture conditions on the fatty acid profile of the oleaginous yeast *R. glutinis* has not been established. Additionally, information regarding the suitable application of the resulting yeast oil based on the physical characteristics is scarce in the literature. To this end, *R. glutinis* was cultured using glucose and (NH₄)₂SO₄ as the main nitrogen source. RSM was employed to establish whether manipulating the culture conditions could increase the level of 18:1 sufficiently to produce a biodiesel with properties akin to rapeseed methyl ester, or whether 16:0 can be suitably increased, producing a lipid more suitable for the care product and food industries.

### Experimental

#### Materials

All chemicals and solvents were purchased from the Sigma-Aldrich Corp. apart from CDCl₃, which was purchased from Fluorochem. All reagents were used as received with no additional purification.

#### Methods

**Microbial cultivation**

*R. glutinis* 2439, purchased from The National Collection of Yeast Cultures (Norwich, UK), was used throughout. The yeast strain was maintained on yeast peptone dextrose agar plates (10 g/l yeast extract, 20 g/l peptone, 20 g/l glucose, 15 g/l agar) at 4°C until used.

For the seed culture, 25 ml of yeast medium (3 g/l yeast peptone, 20 g/l malt extract, 3 g/l yeast extract, 5 g/l peptone, 10 g/l glucose, pH 6.5) was inoculated with a single colony of *R. glutinis*, and was incubated at 28°C and 180 rpm for 24 h. Shaking flask cultures were carried out in 250 ml
Erlenmeyer flasks containing 100 ml medium. The cultures were initiated with 10% (v/v) seeding culture into the original RSM medium (1 g/l yeast extract, 0.1 g/l NaCl, 2 g/l [(NH₄)₂SO₄, 0.4 g/l KH₂PO₄, 0.5 g/l MgSO₄·7H₂O, 0.1 g/l CaCl₂], pH 6.3 and incubated in a rotary shaker at 180 rpm. The resulting biomass was harvested after 120 h of growth.

RSM using a 3³ full factorial design was performed to develop mathematical correlations between three independent variables and to approach the optimum response region [29]. The range of the variables tested was: glucose, 10–30 g/l; (NH₄)₂SO₄, 0.5–1.5 g/l; and temperature, 25–35°C. According to this design, 30 runs were conducted including three replicates at the central point for assessing experimental variance. The 27 experiments are listed in the Supplementary Information. Validation of the cultures was performed at 28°C with:

- 15 g/l glucose, 0.75 g/l (NH₄)₂SO₄;
- 25 g/l glucose, 0.75 g/l (NH₄)₂SO₄;
- 15 g/l glucose, 1.25 g/l (NH₄)₂SO₄;
- 25 g/l glucose, 1.25 g/l (NH₄)₂SO₄.

The final cultures were grown in 4 × 2 l Erlenmeyer flasks containing 500 ml of the RSM medium initiated with 10% (v/v) seeding culture at conditions promoting the desired lipid production. These were: 30 g/l glucose, 0.75 g/l [(NH₄)₂SO₄, 30°C; and 30 g/l glucose, 0.5 g/l [(NH₄)₂SO₄ and 25°C, for high 18:1 and high 16:0.

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<th>Independent variables</th>
<th>Biomass (g/l)</th>
<th>Total lipid (dwt%)</th>
<th>18:1 (%)</th>
<th>16:0 (%)</th>
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<tr>
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<td>Temperature (°C)</td>
<td>Glucose (g/l)</td>
<td>(NH₄)₂SO₄ (g/l)</td>
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<tr>
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<td>x₃</td>
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<td>0(20)</td>
<td>0(1.0)</td>
<td>7.606</td>
<td>15.48</td>
</tr>
</tbody>
</table>

For independent variables, the value given is that used in the model and values in parentheses are the true values.

Dwt: Dried weight; ND: No data; x: Dependent variables; Y: Independent variables.
respectively. Cultures were incubated in a rotary shaker for 5 days at 180 rpm.

The relationship of the variables was determined using the MATLAB® model-based calibration, design of experiments software to fit a radial basis function-multi-quadratic (qRBF) regression analysis to the experimental data. The MATLAB SIMULINK® model was then used to construct a process flow model to predict the outcome of lipid production for different growth conditions. To assess the physical properties of the oils produced, the conditions listed above were replicated at the desired environmental conditions in 2 l flask cultures.

### Microwave extraction

Microwave extraction was undertaken using an Anton Parr Monowave™ 300 microwave reactor equipped with a MAS 24 autosampler capable of loading 10 ml reaction vessels. The biomass was suspended in a 2:1 CHCl3/MeOH mixture (6 ml) with H2SO4 (0.1 ml) and a stirrer bar. The microwave was set on an automated cycle containing: heating to the desired temperature and pressure (typically taking less than 1 min) with 1000 rpm stirring; and the reaction (1–20 min, 1000 rpm stirring); fast cooling using compressed N2 (typically less than 2 min depending on temperature). The resulting oil was extracted into chloroform and washed with water three times. The chloroform was then removed under reduced pressure prior to the analysis.

### Fatty acid methyl ester analysis

The lipid content and fatty acid methyl ester profile were calculated by GC–MS calibrated to known standards.

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>DF</th>
<th>MS</th>
<th>R²</th>
<th>RMSE</th>
<th>CV</th>
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</thead>
<tbody>
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<td></td>
<td>Error</td>
<td>0.07</td>
<td>19.352</td>
<td>3.62E-03</td>
<td>0.979</td>
<td>0.06</td>
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<tr>
<td></td>
<td>Total</td>
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<td>28</td>
<td>0</td>
<td>0.979</td>
<td>0.06</td>
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<tr>
<td>Y1 total lipid</td>
<td>Model</td>
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<tr>
<td></td>
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</table>

OF: Degree of freedom; MS: Mean square; R²: Coefficient of determination; RMSE: Root mean-square error; SS: Sum of squares; Y: Dependent variables.

The GC–MS analysis was carried out using an Agilent 7890A gas chromatograph equipped with a capillary column (60 m x 0.250 mm internal diameter) coated with DB-23 ((50%-cyanpropyl)-methylpolysiloxane) stationary phase (0.25 µm film thickness) and a helium mobile phase (flow rate: 1.2 ml/min) coupled with an Agilent 5975C inert MS detector with triple axis detector. A portion of the biodiesel samples (~50 mg) was initially dissolved in 10 ml dioxane and 1 µl of this solution was loaded onto the column, preheated to 150°C. This temperature was held for 5 min, heated to 250°C at a rate of 4°C/min and then held for 2 min.

### Fuel analysis

The kinematic viscosity was measured with calibrated Cannon-Fenske routine viscometers No. 75 and 150, in accordance with standard test methods set out in ASTM D445 and ISO 3104 at 40°C. Cloud points were measured by cooling the samples by 1°C min⁻¹, holding at each temperature for 10 min and observing any solid formation by eye.

### Results & discussion

#### Regression analysis

Under balanced nutrient conditions, most oleaginous microbes undergo rapid cell proliferation to generate high biomass levels but do not accumulate storage triglycerides. However, severe limitation of a particular substrate causes the channeling of carbon into lipid [30]. In practice, lipid production is normally achieved by limiting the starting concentration of nitrogen in the culture to ensure its exhaustion before the sugar. The relationship between a reduction in nitrogen and lipid content for R. glutinis cultured on glycerol has been established [14]; however, no research to date has examined the effect of nitrogen depletion on the lipid profile of R. glutinis. Similarly, whilst temperature can change the lipid profile for various oleaginous microorganisms [31], this has not been investigated for R. glutinis. Producing a biodiesel substitute requires a high level lipid synthesis in which the predominant ester is 18:1. Similarly, a palm oil substitute requires high levels of 16:0.

We attempted a RSM to discover culture regimes for R. glutinis that would fulfill these requirements. A full factorial design with three factors and three levels, including three replicates of the central point, was used to develop a correlation between temperature, glucose concentration and ammonium sulfate concentration on the production of biomass, total lipid, and production of the fatty acid methyl esters 16:0 and 18:1. Low, medium and high values for the independent variables were coded as -1, 0 and 1, respectively (Table 1).

A regression analysis was carried out to fit the response function and predict the outcomes using a
Optimizing the lipid profile by manipulation of the culture conditions for *Rhodotorula glutinis* | Research Article

qRBF response surface. The results obtained by the qRBF were analyzed by means of the analysis of variance using the 27 experimental data points (Table 2). The total biomass ($Y_1$), lipid content as a percentage of dry weight ($Y_2$), percentage 18:1 ($Y_3$) and percentage 16:0 ($Y_4$) were taken as the dependent variables or responses. Model fit data is shown in Figure 1. The coefficient of determination ($R^2$) of the models for $Y_1$, $Y_2$, $Y_3$ and $Y_4$ were 0.979, 0.987, 0.999, respectively, indicating that the model explains between 97.9–100% of the variability in the response. The root mean squared errors (RMSE) of the models for $Y_1$, $Y_2$, $Y_3$ and $Y_4$ were 0.06 g/l, 1.868% dried weight, 0.632 and 0.487%, respectively, with the predicted sum square error RMSE, a measure of the RMSE calculated after the omission of each data point sequentially to provide an indication of model over-fitting, was acceptable with values of 0.069 g/l, 2.548% dried weight, 1.169 and 0.64%, respectively.

The CV value indicates the degree of precision with which the experiments are compared. In these models, CV values of 12.42, 13.28, 1.04 and 2.63 were observed for biomass, total lipid, percentage 18:1 and percentage 16:0, respectively, signifying that the results were reliable. The probability value (P-value) was also measured for each fatty acid, with values of 0.05, 0.01, 0.001 and 0.01, respectively, indicating statistical significance.

![Figure 1. Response surface model fits for the main fatty acids, lipid content and total biomass. (A) 16:0; (B) 18:1; (C) biomass; and (D) total lipid. Error bars represent the spread of data observed for repeated central point. Squares indicate validation data not included in the construction of the models.](image)

dwt: Dried weight; PRESS: Predicted sum square error; $R^2$: Coefficient of determination; RBF: Radial basis function; RMSE: Root mean squared error.
calculated to ensure that the results were not due to chance occurrence. The P-values of the models were all <0.001, indicating a high significance of the coefficients.

The regression models were employed to develop three-dimensional response surfaces and generated to visualize the combined effects on the dependant variables $Y_1$–$Y_4$ (Figures 2–4) when the effect of two factors was plotted, the other factor was set at level zero as described in Table 2.

### Biomass & total lipid content

The relationship between glucose and biomass production is relatively simple, with greater quantities of biomass produced at higher glucose concentrations (Figure 2). Interestingly, the effect of nitrogen concentration on the amount of biomass produced was minimal, with no effect observed compared with temperature and only slightly more biomass being produced at low nitrogen concentrations compared with the effect of glucose. The effect of temperature on biomass production was significant. Irrespective of the other environmental conditions, growth at 35°C was extremely poor. While *Rhodotorula* sp. have been cultured at temperatures between 20 and 32°C, for most strains the optimal biomass is generally obtained around 28°C [32,33]; this corresponds with the findings of this study, with the optimal temperature lying between 25 and 30°C.

The total lipid, measured as a function of dry weight, was highest at high glucose and low nitrogen concentrations (Figure 2). Previously it has been shown that the higher the carbon-to-nitrogen ratio (C/N), the greater the lipid accumulation [34]. This is due to the up regulation of the enzyme ATP:citrate lyase (an enzyme complex only present in oleaginous microorganisms), which upon nitrogen limitation, put simply, increases the metabolic flux of the carbon source into fatty acid biosynthesis [35]. Interestingly, lipid production is also heavily influenced by temperature. Irrespective of the C/N ratio, very little lipid was produced at high temperatures. The optimal temperature for lipid production is around 30°C, though reasonable levels are obtainable at lower temperatures provided the C/N ratio is sufficiently high (generally, C/N >20) [21]. The very low biomass and lipid yields observed at higher temperatures shows the importance of controlling temperature for lipid production.

### Lipid profile

Producing a suitable feedstock for biodiesel production requires the highest possible 18:1 content. Rapeseed, the predominant biodiesel source in both the EU and China, contains between 55 and 65% 18:1 [36,37]. Temperature-induced variations in the proportions of oleic acid have been previously observed in *Candida oleophila*, *Candida utilis* and *R. toruloides*, but affects were species-specific [19]. In *R. glutinis*, 18:1 production appears favored by high temperatures (Figure 3), with greater than 65% of the lipid being 18:1 at 30°C or above, irrespective of the level of glucose or nitrogen provided in the culture. At lower temperatures (e.g., 25°C), this falls to as little as 45% depending on the other variables. This is in contrast to

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**Figure 2.** Response surfaces for total biomass produced by *Rhodotorula glutinis*. (A) Interaction between glucose and (NH$_4$)$_2$SO$_4$; (B) glucose and temperature; and (C) (NH$_4$)$_2$SO$_4$ and temperature.
Ferrante and Kates, and Granger et al., who reported an increase in polyunsaturated fatty acids at lower incubation temperatures [38,39]. This was demonstrated to be due to an increase in the activity of the Δ12-desaturase enzyme catalyzing the transformation of oleoyl-CoA (18:1) to linoleoyl-CoA (18:2) at lower temperatures. While not as influential as temperature, glucose concentration can affect the lipid profile in the 25–35°C temperature range. As the glucose concentration increases, the percentage concentration of 18:1 decreases inversely with an increase in 16:0. At low nitrogen levels, there was a significant drop in the percentage concentration of 18:1, although this variable does not have as much influence as the temperature. Increasing nutrient limitation has been previously demonstrated to increase the saturated fatty acid composition of the oil produced [20], most likely due to ‘metabolic overflow’ at excess carbon conditions resulting in the channeling of excess citrate into fatty acid biosynthesis [21].

In contrast to biodiesel, producing a palm oil replacement suitable for the care product or food industry requires an enhanced saturated component. Typically, palm oils contain between 35 and 50% saturated components [40–42]. The level of palmitic acid produced by *R. glutinis* is heavily dependent on all the environmental variables. As with 18:1 production, the production of 16:0 was found to be reliant on the temperature with high levels produced at 25°C. A similar relationship between temperature and the degree of unsaturation has been reported for *C. curvatus*, in which the total saturated fatty acid content increased from 43.7 wt% at 34°C, to 54.2 wt% at 22°C [26]. At high glucose or low nitrogen levels, this can reach 30% of the total lipid. The C/N ratio did not have a large effect on the production of the saturated ester. At high glucose concentrations, reasonably high levels of 16:0 were produced irrespective of nitrogen availability. Similarly, at low glucose concentrations, very little 16:0 was formed irrespective of the nitrogen concentration. From this data it is clear that the manipulation of temperature and C/N ratio can alter the lipid profile substantially for *R. glutinis*. It should be noted that, while the relative concentration of the constituents within the mixture may be manipulated in favor of one component or another, an increase in relative concentration does not necessarily equate to a corresponding increase in the mass of that constituent produced. Similarly, a reduced concentration but an increase in yield seen at lower temperatures may be a more desirable growth condition in terms of the total mass of 18:1 or 16:0 produced.

**Validation**

In order to assess the accuracy of the model for predictive purposes, a number of validation tests were performed with measured data compared with values predicted by the models. Validation data points are shown in Figure 1 as solid square markers. In general,
these validation points fall within the range of experimental error for the model demonstrating adequate confidence in the predictions. In order to test the effectiveness of the model for guiding the production of lipids tailored to a particular application, *R. glutinis* was cultured under conditions expected to produce a saturate rich oil (SRG oil) and a monounsaturated oil. The monounsaturated oil was then transesterified with methanol to produce biodiesel (*R. glutinis* methyl ester; RGME). The pertinent properties of the two oils were measured and compared with palm oil and rapeseed methyl ester (Table 3).

While the saturated component of the RGME, of 20%, was reasonably high in comparison to most rapeseed methyl ester (RME) samples, the cloud point remained well within the range found in literature. The kinematic viscosity, which is also affected by a high saturated component, was well within the typical range of most biodiesel fuels, lower than typical RME values and compatible with EN14 214. One major difference between the RGME and RME was the amount of polyunsaturated components. Polyunsaturates are far more prone to oxidation than monounsaturated or saturated esters [43]. While fuels from separate sources are difficult to compare, due to the differing level of antioxidant in the samples, generally biofuels with a higher saturated component are more stable. The lack of polyunsaturated components in RGME further demonstrates the suitability of this source as a fuel substitute.

The main use of palm oil is as a component in care products and in foodstuffs such as chocolate. Both of these applications require a high cloud point and a high viscosity. The saturate rich oil (SRG) was found to have lower overall saturates than generally found in palm oil. However, as there were also less polyunsaturated esters, the viscosity was slightly higher than a typical sample of palm oil. The cloud point was also found to be higher than the typical palm oil, demonstrating the suitability of the SRG oil as a replacement for palm oil in these applications.

**Conclusion**

Empirical models were constructed to predict the impact of culture conditions on yields and the composition of oils produced by *R. glutinis*. By reducing the temperature, while maintaining high glucose in the culture, saturate-rich oil was produced. This oil had a viscosity higher than palm oil and would be highly...
suitable for use in the care product industry. At higher temperatures, with high levels of nitrogen, the oleic acid content could be increased substantially. Transesterification of the oil yielded a biodiesel with properties similar to RME, with excellent low temperature behavior, which is highly suitable as a replacement transport fuel.

**Future perspective**

It is becoming increasingly clear that fuels and chemicals produced from lipids sourced from terrestrial crops are causing large ecological damage, having a negative public image and are in too short supply to meet demand. As such, alternative sources of lipid are becoming increasingly sought. Microbes offer a highly promising source of lipids, though the fatty acid profile, which determines the physical properties, is highly variable and changes dramatically depending on the culture conditions. This has ramifications for whether microbial lipids will slot easily into the current legislative framework, though also offers an opportunity to tailor the fatty acid profile to the desired application. The control and understanding of these processes are important, and only by harnessing this effect will future microbial fuels and chemicals fit easily into the current infrastructure.

**Supplementary data**

To view the supplementary data that accompany this paper please visit the journal website at: [www.future-science.com/doi/full/10.4155/BFS.13.64](http://www.future-science.com/doi/full/10.4155/BFS.13.64)

**Table 3. Comparison of properties of an oleic acid ester rich biodiesel produced from Rhodotorula glutinis (R. glutinis methyl ester) and an alternative oil, rich in saturated esters, produced by R. glutinis (saturated R. glutinis oil).**

<table>
<thead>
<tr>
<th>Properties</th>
<th>RGME (high in 18:1; %)</th>
<th>Rapeseed methyl ester† (%)</th>
<th>SRG oil (high in 16:0; %)</th>
<th>Palm oil† (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0</td>
<td>17</td>
<td>2–5</td>
<td>31</td>
<td>39–50</td>
</tr>
<tr>
<td>18:0</td>
<td>3</td>
<td>0–4</td>
<td>5</td>
<td>3–5</td>
</tr>
<tr>
<td>18:1</td>
<td>67</td>
<td>51–68</td>
<td>46</td>
<td>38–45</td>
</tr>
<tr>
<td>18:2</td>
<td>10</td>
<td>18–25</td>
<td>11</td>
<td>8–12</td>
</tr>
<tr>
<td>18:3</td>
<td>2</td>
<td>7–11</td>
<td>4</td>
<td>Trace</td>
</tr>
<tr>
<td>Density (kg/m³)</td>
<td>933</td>
<td>860–900</td>
<td>879.2</td>
<td>908†</td>
</tr>
<tr>
<td>Kinematic viscosity (mm² s⁻¹, 40°C)</td>
<td>3.46</td>
<td>4.2–4.8</td>
<td>37.3</td>
<td>36*</td>
</tr>
<tr>
<td>Cloud point (°C)</td>
<td>-1.5</td>
<td>-5–1</td>
<td>Room temperature</td>
<td>12†</td>
</tr>
</tbody>
</table>

†The ranges for typical rapeseed and palm oil fatty acid profiles, and RME physical properties were taken from one source [41], though are based on over 44 separate scientific publications.

‡Typical values for palm oil taken from two sources [1,42].

RGME: Rhodotorula glutinis methyl ester; RME: Rapeseed methyl ester; SRG: Saturated R. glutinis.

**Executive summary**

**Background**
- Fatty acid production is temperature and nutrient dependent in most oleaginous yeasts.
- The effect that these factors have on the fatty acid profile, however, is highly dependent on the species and strain.
- Design of experiments was used to optimize specific fatty acid production in *Rhodotorula glutinis*.

**Experimental**
- *R. glutinis* was cultured in 100 ml cultures under a range of conditions and nutrient loadings.
- Design of experiments was undertaken to fit a radial basis function-multiquadratic regression analysis to the experimental data.
- MATLAB SIMULINK® model was then used to construct a process flow model.

**Results & discussion**
- Temperature and nitrogen loading both had a large effect on the fatty acid profile for *R. glutinis*.
- An oil high in monounsaturated esters (67 wt%) comparable to rapeseed oil was produced under high temperature and nitrogen loadings.
- A saturate rich oil (46 wt%), equivalent to palm oil, could also be produced at low temperatures.

**Conclusion**
- The oleic acid rich oil yielded a biodiesel with comparable fuel properties to rapeseed methyl ester.
- The saturated oil had similar properties to palm oil.
- This methodology was highly successful in producing bespoke lipids with tailored properties.

**Future perspective**
- Lipids are fast becoming an important chemical precursor for fuels and higher value chemicals.
- It is important, however, that the lipid profile matches the intended use to assure their compliance with existing international standards.
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No writing assistance was utilized in the production of this manuscript.

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Papers of special note have been highlighted as:
- of interest
- of considerable interest


- Embraces both yeast and algal lipids, with brief descriptions of metabolic engineering approaches for economic viability.
- Covers a wide variety of aspects including kinetic modelling, effects of culture conditions on lipid production and using yeast lipids as a substitute for high-value fats (e.g., cocoa butter).
- Compares the effect of temperature on the fatty acid profile of five yeast species.
- Embraces both yeast and algal lipids, with brief descriptions of metabolic engineering approaches for economic viability.


Optimizing the lipid profile by manipulation of the culture conditions for *Rhodotorula glutinis*

**Research Article**


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