Algal Research 6 (2014) 91–97

Contents lists available at ScienceDirect

Algal Research

journal homepage: www.elsevier.com/locate/algal

# Pyrolysis GC–MS as a novel analysis technique to determine the biochemical composition of microalgae

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ARTICLE INFO

*Article history:*
Received 8 May 2014
Received in revised form 15 September 2014
Accepted 19 September 2014
Available online xxxx

*Keywords:*
Biochemical composition
Microalgae
Analytical method
Py–GC–MS
Pyrolysis

ABSTRACT

The biochemical composition of microalgae is a major factor in the feasibility of microalgae biofuel systems. Currently full compositional analysis entails tedious, costly and time consuming analysis methods. In the current research, an attempt has been made to use Analytical Pyrolysis Gas Chromatography Mass Spectrometry (Py–GC–MS) to determine the biochemical composition of microalgae. By identifying pyrolysis marker compounds of each main biochemical component of microalgae, the composition of algae samples could be estimated. This was aided by performing Py–GC–MS of a model protein, carbohydrate and lipid. Indole was shown to be a decomposition product from the protein fraction and its levels were consistent with the changing protein content. The lipid content of the microalgae could be estimated from the presence of alkanes and the carbohydrate fraction by the presence of 1,2-cyclopentanedione, 3-methyl-. A total of 26 different microalgae and cyanobacteria strains were investigated for their protein, carbohydrate and lipid levels using established analysis techniques. The biochemical compositions are compared to the results from the novel technique using Py–GC–MS and are shown to correspond well; $R^2$ values were found to be 0.6–0.9. The results suggest that Py–GC–MS can be used as a rapid test for studying levels and changes in biochemical composition of different algae using one fast technique with minimal sample preparation.



## 1. Introduction

Microalgae have a vast range of applications in different industries. One of the most researched areas is currently the bio-fuel industry due to the ability of microalgae to produce lipids. Extracted lipids are most commonly converted to fatty acid methyl esters for bio-diesel production. In terms of fuel production the lipid content is the most important factor; researchers often try to tweak growth conditions to achieve the highest lipid accumulation [1,2]. This can involve vast amounts of different growth conditions and sampling points, resulting in a large amount of samples for analysis. Consequentially, this is generally very time and labour consuming when lipid content is determined by lipid extraction techniques.

For biofuel conversion routes such as hydrothermal liquefaction, the levels of carbohydrates and proteins are also of significance as these fractions also contribute to the bio-crude being produced [3]. An estimation of the potential yields and bio-crude quality can only be achieved when the levels of all three of these main biochemical components are known [4]. Proteins are often analysed based on the Lowry assay [5] which involves the use of a Folin reagent, subsequent absorbance measurements at 750 nm and comparison to a protein standard absorbance at this wavelength. Alternately, an approximation method using the elemental composition of microalgae and nitrogen conversion factor can be performed [6]. Recently these two methods have been compared by Laurens et al., 100 samples of three algae strains were investigated. The linear regression between the two protein analysis methods was plotted and resulted in a $R^2$ value of 0.78 [7]. Total carbohydrates are commonly measured using a method which involves hydrolysis using $H_2SO_4$ with phenol being used as a colorimetric indicator for sugars [8]. Alternatively total carbohydrates can be determined by hydrolysis of the carbohydrates to mono-saccharides with subsequent analysis by HPLC [6]. Laurens et al. compared these two techniques and found a correlation of $R^2 = 0.77$. This shows that the methods commonly employed and generally accepted in microalgae research are by no means perfect, even the most commonly used techniques have some discrepancy with $R^2$ values around 0.77 for protein and carbohydrate analyses.

All methods mentioned above involve time-consuming sample preparation, a dry feedstock and are specific to a single analyte. This can pose problems as microalgae accumulate certain biochemical components at different stages of their growth, resulting in vast amounts of sampling and analyses. For example, lipid synthesis occurs during the growth period when nutrients (particularly nitrogen) become depleted [1]. The varying proportions of different biochemical components during the growth cycle are also accompanied with a change in growth

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http://dx.doi.org/10.1016/j.algal.2014.09.009

rate, which must be taken into account when a maximum biomass harvest is desired. Once growth has stabilised this can be exploited to re-inoculate fresh cultures or the biomass can be harvested at this point. Furthermore, it is desirable to know the proportion of the different biochemical components at any given time during growth to maximise recovery of specific components. When the biochemical composition of microalgae is of particular importance, a vast amount of analysis is required to identify different components at different growth conditions. This is usually a tremendously costly and time-consuming process.

The aim of the current research is to demonstrate an analytical method for multiple biochemical components using a single, simple technique with minimal pre-treatment. Pyrolysis Gas Chromatography Mass Spectrometry (Py–GC–MS) is explored as a technique to rapidly estimate microalgae composition. Analytical pyrolysis has previously been used to study the seasonal variation of seaweed as a bioenergy feedstock by Adams et al. [9]. Marker compounds were assigned to carbohydrate, lipid, protein and phenolic origins of the macroalgae to enable qualitative comparison of the composition of macroalgae over its yearly growth cycle.

The need for improved and faster analysis techniques for the composition of microalgae samples is evident from recent research into Fourier-transform infrared spectroscopy. Mayers et al. recently showed that FTIR could be applied to dried samples of *Nannochloropsis* for the determination of the main biochemical components [10]. Their data showed very good correlation compared to conventional protein, lipid and carbohydrate analysis. Wagner et al. were able to perform similar research on microalgae composition using FTIR with concentrated algae cells in suspension [11]. Their research equally showed good correlation to traditional analysis methods. The methodology presented in the current research differs somewhat from the FTIR approach as that even less sample is required (<0.1 mg compared to 3–5 mg), is applicable to a wide range of microalgae species, not affected by high ash contents and has the potential of identifying levels of additional compounds apart from the three main biochemical components.

Kebelmann et al. previously performed pyrolysis experiments on three microalgae strains by Py–GC–MS and thermo-gravimetric analysis (TGA). They were able to show the distinct differences of pyrolysis products from proteins and lipids [12]. The compounds detected from Py–GC–MS were assigned to their biochemical origin, for example indole and toluene were derived from the protein fraction and hydrocarbons such as heptadecane from the lipid fraction. It was concluded that pyrolysis of microalgae produces a range of useful products for bio-fuels. The technique was however not used as a way of characterising the original feedstock composition. Valdés et al. performed TGA and Py–GC–MS analysis on three samples of *Nannochloropsis occulata* and were able to show a correlation between the sum of pyrolysis products from different biochemical products and traditional analysis techniques [13]. A total of 20 pyrolysis products in chromatograms were assigned to protein, lipid or carbohydrate origin in their work. The sum of these markers showed a clear correlation to the analysis carried out by established techniques. This approach appeared to work well for a limited sample set of one algae strain but still involved a considerate amount of data analysis. The current paper investigates a similar technique with a large sample set from different algae strains and a novel data analysis approach.

## 2. Materials and methods

Pyrolysis–GC–MS analysis was performed using a CDS 5000 series pyrolyser (CDS Analytical Inc., Oxford, PA, USA) connected to a Shimadzu 2010 GC–MS (Shimadzu Corporation, Kyoto, Japan). Samples of approximately 0.1–0.5 mg were weighed into a pre-weighed quartz tube (25 mm length and 0.5 mm inner diameter) with quartz wool at one end. Subsequently the other end of the tube was filled with quartz wool to keep the sample in place. The tube was reweighed before pyrolysis. Pyrolysis was performed at a temperature of 500 °C with a ramp rate of 20 °C/ms with a hold time of 20 s. The volatiles were trapped on a TENAX adsorbent trap before being desorbed at 300 °C onto a heated transfer line which was held at 300 °C. The purge flow of helium to remove any oxygen from the sample, prior to pyrolysis, was set to 20 ml/min. The heated transfer line was connected to the split/splitless injector of the GC inlet port which was set to 280 °C. The split ratio was set to 20:1. The pyrolysis products were separated on an Rtx 1701 60 m capillary column, 0.25 id, 0.25 μm film thickness, using a temperature programme of 40 °C, hold time 2 min, ramped to 280 °C (6 °C/min), hold time 15 min and a constant column head pressure of 2.07 bar. After pyrolysis the tube was reweighed to determine the amount of sample pyrolysed. This allowed calculation of normalised peaks as an area per mg sample pyrolysed. The mass spectrometer ion source was set to 230 °C and the interface to 280 °C, scanning took place once per second in the range of 50 to 550 *m/z*. Peaks were identified using the NIST mass spectral database versions 147 and 27 with an identity threshold cut-off of 50.

Py–GC–MS was performed on 26 different microalgae samples. The strain names and growth conditions are listed in Table 1. All were freeze dried before analysis; no further sample preparation was performed. Additionally model compounds of protein, lipids and carbohydrates were analysed. Bovine albumin serum was used as a protein standard and purchased from Sigma-Aldrich (#B4287). Starch (Sigma-Aldrich #179930) and the monomer sugars glucose (Sigma-Aldrich #G8270), xylose (Sigma-Aldrich #1500) and mannose (Sigma-Aldrich #M2069) were investigated as carbohydrate standards. As a lipid standard sunflower oil, palm oil from commercial sources and palmitic acid (Sigma-Aldrich # P0500) were pyrolysed. Chlorophyll *a* was extracted from fresh spinach using acetone/water (90:10% v/v) and astaxanthin was purchased from Santa Cruz Biotechnology, Inc. (#SC-202473).

The microalgae samples were analysed by TGA for ash and moisture content on a TA Instrument IR5000Q TGA in air. The TGA oven temperature was increased to 105 °C and held for 15 min, subsequently ramped to 550 °C and held for 80 min to determine the moisture and ash contents respectively. The air flow inside the TGA furnace was set to a 50 ml/min.

The biochemical composition of the microalgae strains were determined by traditional techniques; for the protein analysis, a modified Lowry method by J. Waterborge was used [14] which involves the use of a Folin reagent, subsequent absorbance measurements at 750 nm and comparison to a bovine standard absorbance at the same wavelength. For this procedure 50 mg of the sample was weighed and mixed with 5 ml 2 M NaOH in a sample tube and placed in a beaker of boiling water for 10 min. Subsequently the tube was centrifuged for 5 min and 2.5 ml of the supernatant was pipetted into a fresh tube containing 7.5 ml 2 M NaOH and mixed. 0.5 ml of this solution was mixed with 5 ml of a complexing reagent made up of copper sulphate and sodium potassium tartrate solutions and left for 10 min. Finally 0.5 ml of Folin reagent was added to the sample and the absorbance was measured at 750 nm after zeroing with a reagent blank.

Total carbohydrate content was determined by the sulphuric acid hydrolysis method [8]. This involved weighing approximately 50 mg of sample (and glucose as a standard) into a 10 ml sample tube. 3 ml of 72 wt.% sulphuric acid was added, mixed and placed into an oven at 40 °C for 30 min. After hydrolysis, samples were diluted to 100 ml and spun at 3500 rpm for 5 min (*g* force of 2264). 1 ml of the supernatant was added to two separate tubes, one with 1 ml 5 wt.% phenol solution and one with 1 ml distilled water (sample blank). To all tubes 5 ml of conc. sulphuric acid was added, mixed and left for 1 h. The spectrophotometer was set to 485 nm and zeroed with a reagent blank and absorbance was measured for sample blanks and samples.

Lipid extraction was performed using a modified Folch method, employing a 2:1 chloroform/methanol extraction at 35 °C using an ultrasonic bath for 45 min [15]. Approximately 100 mg of algae was treated with aliquots of 5 ml of solvent mixture. After sonication, 1.7 ml of

**Table 1**
Biochemical analysis on a dry ash free basis of microalgae data set based on traditional techniques.

| Algae name | Condition | Protein (%) | Error ± | Carbs (%) | Error ± | Lipid (%) | Error ± | Ash (%) | Moisture (%) |
|---|---|---|---|---|---|---|---|---|---|
| *Scenedesmus obliquus* | Raceway, day 8 batch 1 | 48.8 | – | 25 | 1 | 22.5 | – | 12.9 | 7.5 |
| *Scenedesmus obliquus* | Raceway, day 8 batch 2 | 45.6 | – | 19.2 | *0* | 19.5 | – | 17.6 | 3.9 |
| *Scenedesmus obliquus* | Raceway, day 22 batch 1 | 44.4 | – | 26.5 | – | 20.1 | – | 12.4 | 3.2 |
| *Scenedesmus obliquus* | Raceway, day 22 batch 2 | 42.9 | – | 25.3 | *2* | 21.8 | – | 12.3 | 3 |
| *Scenedesmus obliquus* | Raceway, standard media | 17 | – | 46.1 | *1* | 19.2 | – | 5.4 | 2.4 |
| *Scenedesmus obliquus* | Raceway, standard media stressed | 11.1 | – | 40.7 | *1* | 25.7 | – | 4.9 | 4.3 |
| *Scenedesmus obliquus* | Raceway, unknown | 36.1 | *1.8* | 28.3 | – | 16.9 | *0* | 7.1 | 5.0 |
| *Pseudochoricystis ellipsoidea* | Low nitrogen media | 20 | *1.5* | 35.6 | *0* | 34.3 | – | 0.7 | 2.5 |
| *Pseudochoricystis ellipsoidea* | High nitrogen media | 51.8 | *3.2* | 25.7 | *1* | 25.7 | *0* | 2.2 | 5.2 |
| *Pseudochoricystis ellipsoidea* | Photobioreactor, unknown media | 10.2 | – | 34.1 | 2 | 38.1 | – | 7.4 | 1.7 |
| *Pseudochoricystis ellipsoidea* | Outdoor raceway, centrifuged | 27.5 | *1* | 19.3 | *0* | 45.4 | *0* | 2.3 | 2.6 |
| *Pseudochoricystis ellipsoidea* | Outdoor raceway, chem. flocculation | 24.9 | *1.3* | 22.2 | *2* | 36.7 | *0* | 8.8 | 4 |
| *Chlorogloeopsis fritschii* | Photobioreactor, unknown media | 41.8 | *0.3* | 37.8 | *2* | 8.2 | *0* | 4.6 | 5 |
| *Chlorogloeopsis fritschii* | Photobioreactor, unknown media | 35.1 | *0.9* | 23.9 | *0* | 8.4 | *0* | 7 | 5.7 |
| *Chlorella vulgaris* | Glucose media heterotrophic | 33.9 | *0.3* | 45.8 | *1* | 4.2 | *0* | 7.1 | 6.5 |
| *Chlorella vulgaris* | Sucrose media heterotrophic | 36.7 | *1.4* | 50.1 | *3* | 5.6 | *0* | 17.9 | 9.0 |
| *Chlorella vulgaris* | Molasses media heterotrophic | 41.6 | *2* | 35.9 | *1* | 7.2 | *1* | 11.6 | 11.5 |
| *Chlorella vulgaris* | Glycerol media heterotrophic | 17.6 | *0.4* | 60.4 | – | 23.2 | *1* | 7.8 | 15.4 |
| *Chlorella vulgaris* | Unknown phototrophic | 45.8 | *0.8* | 36.4 | *1* | 14.5 | *4* | 11.7 | 4.4 |
| *Chlorella vulgaris* | Unknown phototrophic | 53.1 | *1.3* | 23.6 | *1* | 15.3 | *1* | 5.5 | 5.8 |
| *Chlorella vulgaris* | Unknown | 10.4 | – | 12.7 | 1 | 58.0 | – | 8.2 | 1.5 |
| *Chlorella FC2 IITG* | Unknown | 10.4 | – | 24.5 | 2 | 37.3 | – | 4.3 | 1.7 |
| *Chlorella vulgaris minutissima* | Unknown | 10.3 | – | 13.9 | 6 | 56.7 | – | 3.3 | 1.5 |
| *Chlorella emersonii* | Unknown | 9.03 | – | 37.9 | 2 | 29.3 | – | 2.8 | 3.1 |
| *Chlorella zofingiensis* | Unknown | 11.2 | – | 11.5 | 1 | 56.7 | – | 4.8 | 1.6 |
| *Spirulina sp.* | Unknown | 50.1 | *4.5* | 15.1 | *0* | 12.3 | *0* | 7.6 | 5.7 |

water was added to the samples, centrifuged at 2000 rpm for 10 min and the lipid containing chloroform layer recovered. The procedure was repeated with fresh solvent mixture. The lipids were finally quantified gravimetrically.

## 3. Results and discussion

### 3.1. Microalgae analysis

All 26 freeze-dried microalgae strains were analysed by the traditional analysis techniques for protein, carbohydrate and lipid contents as outlined above, the results are presented in Table 1. All values are presented on a dry ash free basis. Although the newly proposed Py–GC–MS method does not measure the inorganic fraction of the microalgae samples, the amount of material pyrolysed is related to the organic fraction of the algae. A high inorganic fraction in algae, results in a lower pyrolysis signal from a smaller percentage of algae being pyrolysed compared to a low ash sample. Due to the MS response being normalised to the amount of algae pyrolysed the results are related to the organic fraction of the algae which is why we are comparing the results from the new method to the dry ash free biochemical composition.

The strains investigated include seven strains of *Scenedesmus obliquus* obtained from the University of Bath, UK. Different growth conditions were used for each sample. The results from the traditional analysis techniques show a range in biochemical composition for *Scenedesmus*; protein levels range from 11–48%, carbohydrates from 19–46% and lipid levels are in a narrow range of 17–26%. The data set also contains eleven samples of *Chlorella*, four strains were grown heterotrophically with different organic carbon substrates at the University of Leeds and five were grown phototrophically at the University of Bath. The *Chlorella* samples have a very broad range of biochemical compositions ranging from 6–58% lipid content. Additionally, three strains of *Pseudochoricystis ellipsoidea* from the DENSO COROPORATION grown in photobioreactors, two strains of *Chlorogloeopsis fritschii* from the Plymouth Marine Laboratory and one sample each of *P. ellipsoidea* grown in outdoor raceway ponds in Japan (DENSO COROPORATION, Japan). One was harvested by centrifuge; the other was harvested by chemical flocculation. The average composition of all algae lies at 30% protein, 30% carbohydrates and 26% lipids. Each analysis was carried out in duplicate, when sufficient algae mass was available for duplicate analysis, and average values are reported. The measurement error range for the protein, carbohydrate and lipid analyses, lie at 1.5, 1.3 and 0.5% respectively. Therefore, we report the single, non-duplicate values with relatively high confidence.

### 3.2. Py–GC–MS analysis of model compounds

Model compounds were pyrolysed at 500 °C and the chromatograms were investigated to identify unique pyrolysis marker compounds. Peaks specific to model compounds, which do not appear on the chromatograms of other compounds, were selected. These peaks were subsequently identified in the chromatograms of microalgae. By comparing peak sizes of the unique marker compounds and normalising to the amount of algae pyrolysed, the amount of each respective model compound present in the microalgae samples could be calculated. The main pyrolysis products of all model compounds investigated are presented in Supplementary Table S1.

The chromatogram of the pyrolysis products from bovine serum is plotted in Fig. 1. The marker compound selected to represent the protein fraction from microalgae is *Indole*. This compound was selected as it was not identified in any other structural model compounds investigated and had a relatively large peak area in the pyrolysis chromatogram. The total percentage area of the *Indole* peak is 4.3%. Toluene (14.2%) was also found to be a major product of bovine serum pyrolysis but this compound was also detected as a pyrolysis product of pigments.

Palmitic acid (C16:0) is the most abundant fatty acid in nature and was investigated as a model compound for microalgal lipids. Using palmitic acid is an oversimplification as the fatty acid profiles of microalgal lipids are much more complex than a single fatty acid. The majority of lipids in microalgae are typically present as triglycerides and some free fatty acids stored in the cell chloroplasts, but fatty acids are also present as membrane lipids such as glycolipids and phospholipids [16]. The total lipid content determined by the Folch method includes membrane lipids and suffers from overestimation by extracting carotenoids. During our pyrolysis investigation we found that pigments such as chlorophyll a and astaxanthin, which are extracted by the Folch method, produced alkanes and fatty acid methy esters and alcohols. Sunflower oil and palm oil were also pyrolysed to compare pyrolysis

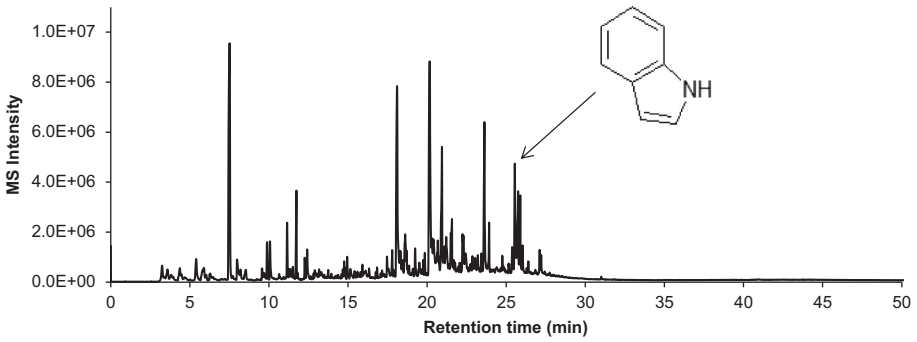



**Fig. 1.** Py–GC–MS total ion chromatogram of bovine serum at 500 °C.

products from triglycerides and fatty acids. The main products detected from both fatty acids and triglycerides were alkanes. Pyrolysis of a fatty acid with a particular chain length led to the formation of alkanes with varying chain lengths. Most alkanes detected in chromatograms were one carbon link shorter than the original fatty acid. Alkanes with 2 or 3 fewer carbon links were also found in decreasing quantity. Heptadecane was found in all chromatograms of microalgae and was therefore chosen as the lipid marker compound. Pigments also produce alkanes at the pyrolysis conditions used (see Supplementary Table S1), although they are produced in much lower concentrations than those derived from lipids. The methodology therefore will lead to an overestimation of lipid content compared to common GC/FAME analysis [17] which only quantifies the biofuel potential of microalgae based on the acyl chains of the lipids. The Folch method used in the current work is suitable for the research as it includes all types of lipids and the Py–GC method equally includes them. The methodology should not be regarded as fully quantitative but it provides a reasonable estimation of the lipid content of algae.

For the analysis of carbohydrates, starch was used as a model compound. This is again a simplification for total microalgal carbohydrates as not all microalgal carbohydrates are starch. Carbohydrates in microalgae serve two main functions: as a structural component of the cell wall and as storage carbohydrates for energy. Carbohydrates are made up of varying components including simple sugars (monosaccharides) and their polymers (di- and poly-saccharides). Different algal species tend to accumulate different types of carbohydrates. Cyanobacteria mainly synthesise glycogen (α-1,4 linked glucans), red algae synthesise floridean starch (hybrid of starch and glycogen) and green algae synthesise amylopectin-like polysaccharides (starch) [18]. This leads to a different profile of mono-saccharides when hydrolysed carbohydrates are analysed. The most abundant sugars found in microalgae are glucose, galactose, xylose, mannose and rhamnose [18]. There are typically also small amounts of cellulose and alginates present, therefore, using starch as a model compound for total microalgal carbohydrates likely leads to an underestimation.

1,2-Cyclopentanedione, 3-methyl- was chosen as a marker compound as it was found in all of microalgae samples and not in any other model compounds investigated. Furfural and levoglucosenone were also likely candidates but could not be identified in all strains investigated. A summary of the marker compounds, their structure and Kovats retention index are presented in Table 2.

### 3.3. Py–GC–MS analysis of microalgae

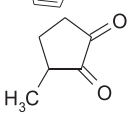
Fig. 2 shows a typical chromatogram of microalgae. Specifically, the plot shows the chromatogram of *S. obliquus* grown in nitrogen limited media, pyrolysed at 500 °C. The three main marker compounds from Table 2, previously identified from model compounds, are indicated. Marker compound peaks appear considerably smaller for microalgae compared to the respective model compound chromatograms. The areas of marker compound peaks were calculated as an absolute area by dividing peak area by the mass of sample pyrolysed. These area/mg values are then compared to other samples allowing comparison of specific compound concentrations on a dry ash free basis.

### 3.4. Correlation of Py–GC–MS to traditional methods

#### 3.4.1. Protein analysis

The protein content of the 26 microalgae strains was analysed using the Lowry method and plotted in Fig. 3a on the X-axis. The same microalgae samples were also pyrolysed using the analytical pyrolyser and GC–MS. The peak areas of *Indole* were quantified and divided by the mass of sample pyrolysed (mg) in order to normalise the data points between samples. This resulting peak area value was plotted on the Y-axis and a linear relationship between the two analysis techniques was calculated. The linear regression of the two methods is shown to fit very well with a $R^2$ value of 0.8. This value surpasses that obtained by comparing two traditional and commonly accepted methods for protein analysis presented by Laurens et al. [7]. The equation provided, now allows researchers to estimate the absolute protein content of any microalgae sample with reasonable accuracy. The advantage of this

**Table 2**
Marker compounds identified for each biochemical component.

| Biochemical component | Marker compound | Structure | Kovats retention index* |
|---|---|---|---|
| Protein | Indole |  | 1043 iu |
| Carbohydrate | 1,2-Cyclopentanedione, 3-methyl- |  | 1304 iu |
| Lipid | Heptadecane |  | 1700 iu |

* Data from NIST spectral database 27 on BPX-5 column.

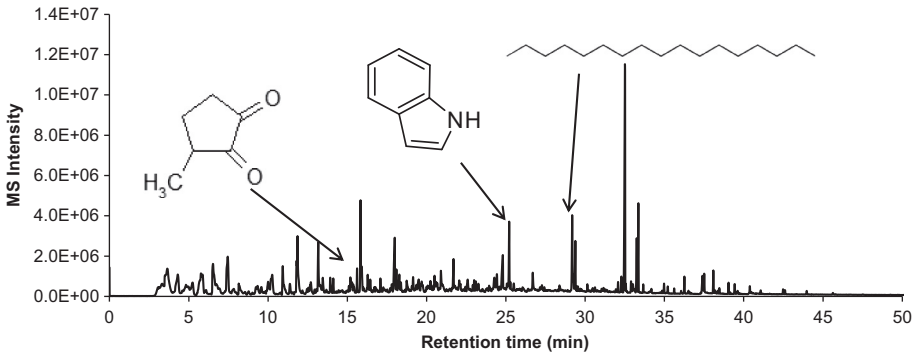



**Fig. 2.** Chromatogram of *Scenedesmus* (standard media, stressed) with marker compounds indicated.

technique over others is that only 0.1 mg of sample is required. This is beneficial when microalgal growth trials are conducted on small scale and the scientist wants to know the change in protein content over time. Fig. 3b shows the data points for *Scenedesmus* and *Chlorella* samples only. It is expected that the same strains of microalgae have an improved relationship when this novel technique is applied. The data shows that $R^2$ values now increase slightly compared to when the entire range of microalgae samples are investigated. This suggests that different amino acid profiles of different microalgae strains could have an effect on this proposed analysis technique. It is therefore beneficial to have a unique linear equation for each microalgae strain. Nevertheless this simple technique is expected to allow a quick estimation of any microalgae strain, even though only 26 different samples were investigated.

### 3.4.2. Carbohydrate analysis

Fig. 4a presents the data of total carbohydrate analysis from all microalgae samples investigated. The data from Py–GC–MS was calculated in the same way as for protein analysis but with the peak areas of 1,2-cyclopentanedione, 3-methyl-. It can be seen that the two analyses have a clear corresponding trend but the $R^2$ value is lower compared to the above protein analysis. The $R^2$ value is 0.61 and some data points deviate significantly from the linear trend line. For example, the Y-axis data point at around 34% carbohydrates is lower than the one at 28%. It appears that the different microalgae samples do not produce the same amount of 1,2-cyclopentanedione, 3-methyl-, depending on their carbohydrate content based on the acid phenol method. This is most likely due to the compound, 1,2-cyclopentanedione, 3-methyl-, being chosen on the basis of the model compound starch. As discussed above microalgae also contain other carbohydrate structures which most likely leads to the discrepancies in the two methodologies. Nevertheless a linear trend is observed in the two techniques and the method can give a quick estimation of carbohydrate content.

Similarly to Fig. 3b, Fig. 4b presents the data points of the seven *Scenedesmus* and eleven *Chlorella* samples. The linear regression lines now show a better correlation to the entire microalgae data set. This supports the above hypothesis that different carbohydrate compositions in different microalgae strains have an effect on the correlation of the two techniques. For this purpose it would be beneficial to investigate the structural composition of the carbohydrate fraction of the

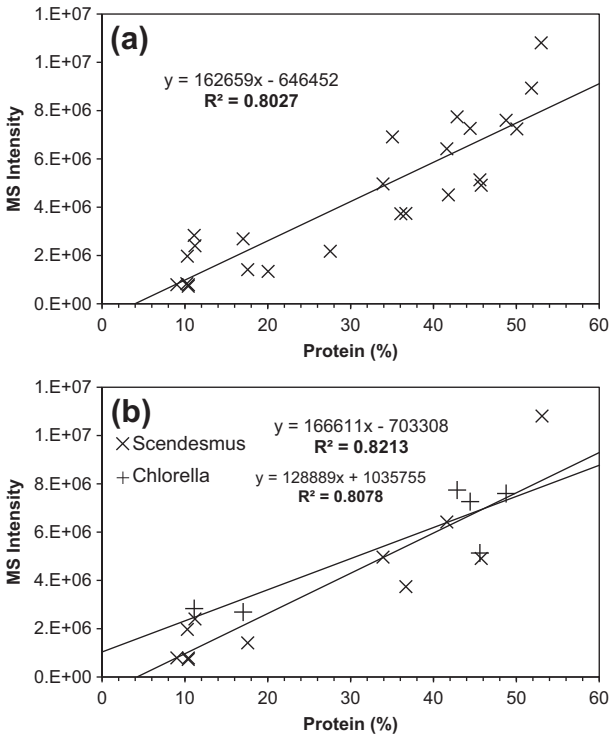



**Fig. 3.** Correlation of Py–GC–MS protein analysis to traditional Lowry protein determination; (a) entire data set, (b) *Chlorella* and *Scenedesmus* samples only.

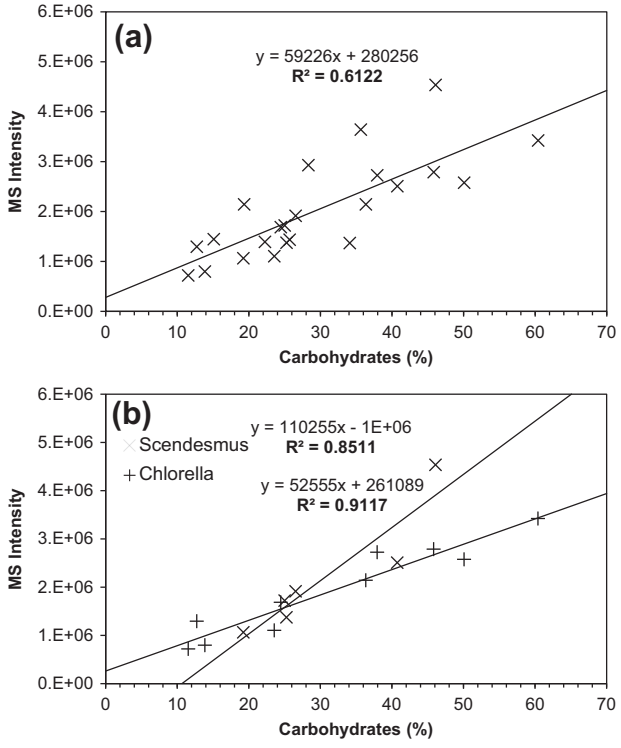



**Fig. 4.** Correlation of Py–GC–MS carbohydrate analysis to traditional carbohydrate determination; (a) entire data set, (b) *Chlorella* and *Scenedesmus* samples only.

microalgae samples and compare the pyrolysis products. This could lead to a more accurate overall linear relation for any microalgae sample but also potentially shed light on the carbohydrate composition of microalgae by simply investigating the pyrolysis products. For the separate linear regression lines the $R^2$ values increase from 0.6 to 0.85–0.91 which represents a very good correlation of the two analysis techniques.

### 3.4.3. Lipid analysis

Total lipids were measured using the Folch method as described previously. The results for all 26 strains of microalgae are plotted on the X-axis in Fig. 5a. The algae range from very low levels (~5%) to very high levels above 55%. Each microalgae was pyrolysed and the peak areas for heptadecane quantified and normalised. These normalised areas are plotted on the Y-axis in Fig. 5a. The linear correlation between the two methods resulted in a $R^2$ value of 0.65. The $R^2$ value is reasonably good for such a unique method. It can be seen however that some values are largely overestimated, especially the two samples at around 57% lipids. Equally there are samples which were underestimated, notably the 45% lipid sample. Over and under estimations are most likely due to different fatty acid profiles or different lipid classes amongst different microalgae samples. Using just one alkane as a marker compound rather than the sum of all alkanes is likely the cause of the deviance. Nevertheless a clear general trend between the two analysis methods can be seen and a relatively good $R^2$ value is obtained. The method could likely be improved by taking the sum of all alkane peaks observed in chromatograms. This presents a trade-off between accuracy and simplicity and speed. In the course of the current work the aim was to keep the data analysis as simple as possible for high throughput.

Fig. 5b shows the data of two strains of microalgae, namely the seven *Scenedesmus* samples and eleven *Chlorella* samples. The larger data set and broader lipid distribution of *Chlorella* samples result in good linear relation between the two methods; a $R^2$ value of 0.82 is achieved. The seven *Scenedesmus* samples have a much narrower range of only 10% difference in lipid content and result in a worse fitting linear relation, $R^2$ is 0.69. Considering how close the lipid levels are clustered together for *Scenedesmus*, the average lipid analysis error of ±0.7, shown in Table 1, could have a significant impact on the $R^2$ value in this analysis. Another reason could be the variance in analysis of repeats in the Py–GC method itself, which is addressed in the subsequent section.

### 3.4.4. Repeatability of Py–GC-method

Two samples were analysed multiple times using the Py–GC method, a *S. obliquus* sample and a *Chlorella vulgaris* sample. The *Scenedesmus* sample exhibited 36.1% protein, 28.3% carbohydrates and 16.9% lipid using the traditional analysis techniques; the *Chlorella* sample exhibited 53.1% protein, 23.6% carbohydrates and 15.3% lipid. The samples were analysed by Py–GC in triplicate. The MS intensity results are plotted in Fig. 6 along with the trend lines derived from the experiments in previous sections. It can be seen that the majority of replicate data points are very close together. The only data points where repeat analysis deviates significantly are for the protein levels of *Chlorella* determined by the conventional method to be 53%. Using the equation derived for protein content in Section 3.4.1 ($y = 162659x - -646452$), the protein content is calculated as 74.3, 82.9 and 75.04% respectively. This equates to a standard deviation of 4.7 and deviation of the mean 77.4 ± 2.2. This deviation is low, showing the high repeatability of the analysis. However, the estimation of the protein content using the equation is significantly higher (by 22%) than that measured by the Lowry method. The improved regression line using the data for *Chlorella* samples only, in Fig. 3b ($y = 128889\times x + 1035755$), results in an average of 84% protein, still around 23% too high. However, this is the worst case scenario observed in the current work. For example the calculated lipid content using the Py–GC method agrees within 2% of the Folch method's observed value. The deviation of repeats for carbohydrates and lipid for *Chlorella* are calculated as 1.3 and 0.3 respectively.

For the repeat analyses of *Scenedesmus* the deviations from the calculated mean for protein, lipid and carbohydrate contents are 0.9, 0.2 and 1.5 respectively. These results show that the Py–GC method has a high repeatability in itself, however calculating the biochemical contents using the equations derived, can lead to large over or underestimations as shown in examples in previous sections. Nevertheless the linear regressions fit well for the majority of the samples, especially for individual algae species. Therefore the newly proposed technique can be a very beneficial tool in showing trends of biochemical compositions of unknown microalgal samples. This is especially useful during growth trials where small amounts of sample are available, the results from Py–GC analysis can then be used to choose which samples to scale up.

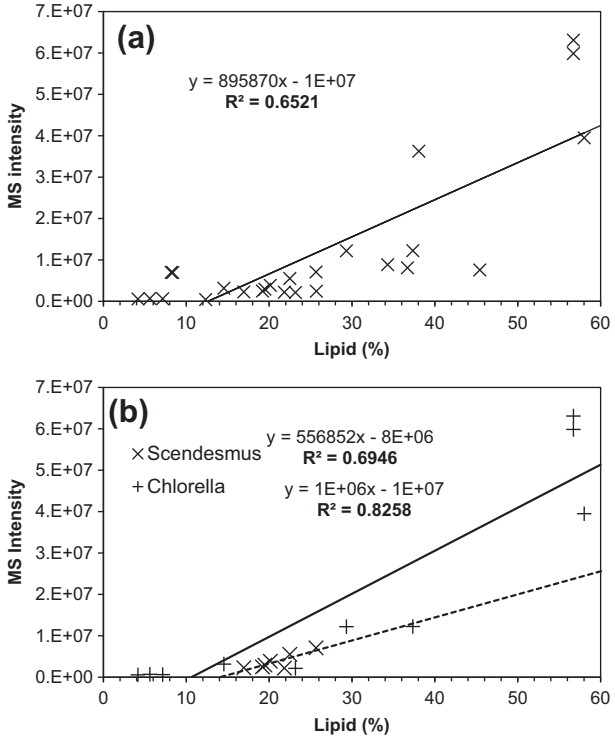



**Fig. 5.** Correlation of Py–GC–MS lipid analysis to traditional lipid Folch determination; (a) entire data set, (b) *Chlorella* and *Scenedesmus* samples only.

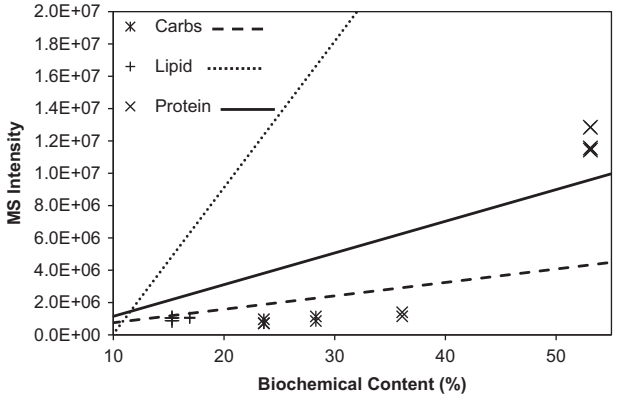



**Fig. 6.** Repeat data point analysis for *Scenedesmus* and *Chlorella* with regression lines for protein, carbohydrates and lipids.

## 4. Conclusions

This work presents a novel technique for the fast analysis of microalgae biochemical composition. The main advantages of the proposed technique are:

1. Fast analysis for thee parameters (~1 h).
2. Minimal sample amount required (<0.1 mg).
3. No toxic or harmful chemicals.
4. Minimal sample handling.
5. Only one piece of equipment required for the full biochemical characterisation.

It is shown that by identifying the peak areas of three marker compounds for the main biochemical components, lipids, carbohydrates and proteins, the levels of these components can be estimated in the original biomass. Protein levels could be determined with a good correlation to the Lowry method of $R^2 = 0.8$ over the entire data set. The technique showed improved correlation when only data points from separate microalgae strains were investigated. This suggests that the method can faithfully predict the microalgal composition during growth trials from one strain at different conditions or growth stages. Py–GC–MS lipid analysis was compared to the Folch method and achieved a correlation of $R^2 = 0.65$. The carbohydrate analysis achieved a correlation of $R^2 = 0.61$, most likely due to the difference in different microalgae strain carbohydrate composition. Individual carbohydrate algae strain correlation was as high as $R^2 = 0.91$. Overall this work presents a fast and easy technique for microalgae researchers when a vast number or sample points in growth trials are analysed. The novel technique is not as accurate as established techniques but gives a reasonable estimation of lipid, protein and carbohydrate contents of microalgae in one easy step.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.algal.2014.09.009.

## Acknowledgments

The authors would like to thank Simon Lloyd for his technical support. The authors are also grateful to Dimitrios Kaloudis and Philippe Mozzanega from the University of Bath for their provision of the *Scenedesmus* and *Chlorella* biomasses. Gratitude is also extended to The Plymouth Marine Laboratory and the DENSO CORPORATION for provision of microalgal samples. Finally the authors would like to thank the EPSRC for financial support (EP/I014365/1) and the Doctoral Prize Fellowship for Dr Patrick Biller (EP/L504993/1).

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