FTIR transmission spectroscopy for measurement of algae neutral lipids

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Abstract: Algae lipids can be used to produce biofuels and are considered a potential source of energy to supplant fossil fuel. Cultivation practices of algae grown in large ponds can be tailored to maximize lipid content. Laboratory methods of measuring lipid content are time-consuming and labor-intensive, so a real-time measuring technique is needed to efficiently control the addition of pond nutrients. The objective of this research was to determine the effectiveness of measuring algae lipid content with Fourier Transform Infrared (FTIR) transmission spectroscopy. Six algae samples (*Nannochloropsis salina*) with varying lipid contents were centrifuged and then dried in an oven at 40 °C for 12 hours. Dried algae were mixed with potassium bromide (KBr) powder at a mass ratio of 1:150 (algae: KBr) and pressed into pellets. A Thermo-Nicolet 6700 FTIR spectrometer was used to collect spectral data in transmission mode. Three relevant absorption bands centered at 2920, 2855, and 1742 cm^{-1} were identified. A linear regression analysis showed that the band depth at 2920 cm^{-1} was strongly correlated (R^2 = 0.92) with lipid content measured by gas chromatography (GC). The results of this research provide insight into the development of a real-time lipid-content sensor.

Keywords: biofuels, FTIR spectroscopy, ATR spectroscopy, KBr pellets, Mid-infrared spectroscopy

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1 Introduction

Biofuels are derived from living organisms like plants, animals, and algae. Interest has emerged in recent years in using biomass-derived fuels to mitigate the depletion of fossil fuels in light of the world's increasing energy demands (Demirbas, 2010; Kosaric and Velikonja, 1995; Brune et al., 2009). Microalgae have high biomass and lipid productivity compared to other biomasses (Chisti, 2007; Han et al., 2011), and some species have lipid contents constituting about 50%-60% of their dry weight (Jones and Mayfield, 2012). Algae are capable of yielding more biofuel than other feedstocks per unit of growing area (Gao et al., 2012; Scott et al., 2010). A proposed process of producing biofuels from algae involves cultivating algae in large ponds and adding nutrients in ways that enhance lipid production, harvesting and extracting lipids, and converting lipids into biodiesel through transesterification (Sauli and Sarbatly, 2012).

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Temperature, nutrient availability, and light intensity in a given algae culture are all factors affecting the content and composition of the lipids (Converti et al., 2009; Li et al., 2010). Another important factor involves selecting high-yield microalgae strains (Griffiths and Harrison, 2009). Thus, lipid-measurement techniques are needed for process control and varietal screening.

Current laboratory-based methods for measuring lipids in algae include chemical extraction and gas chromatography (GC), both of which are time-consuming and labor-intensive (Dubé et al., 2004). Rapid, continuous measurement methods need to be developed for online systems in algae ponds or biofuel production facilities. Han et al. (2011) described six different methods of lipid-content analysis including: (1) gravimetric quantification, (2) staining, (3) calorimetric sulfo-phospho-vanillin (SPV), (4) time domain nuclear magnetic resonance (TD-NMR), (5) thin layer chromatography (TLC) and high-performance liquid chromatography (HPLC), (6) and near-infrared (NIR) and Fourier transform infrared (FTIR) spectra methods. Gravimetric quantification is the most common and accepted method, but it is time consuming, requires a large number of samples, and is labor-intensive. Staining requires fluorescent lipophilic dyes like Nile red and boron-dipyrromethene (BODIPY) (Cooksey et al., 1987; Govender et al., 2012), which selectively adhere to neutral lipids in algae cells. Fluor spectrophotometers are used in conjunction with staining to measure the fluorescence intensity of the staining dyes. The disadvantage of this method is the uneven dye uptake resulting from differences in cell wall composition among different algae strains and growing conditions (Gao et al., 2008; Laurens and Wolfrum, 2011).

In calorimetric SPV, sulfuric acid and vanillin-phosphoric acid reagent are added to the algae sample for color development, and the lipids are indirectly measured by taking an absorbance measurement at 540 nm (Cheng et al., 2011). Though the preparation time required before sample analysis can be lengthy, this method requires less time and labor when a large number of samples is analyzed. The TD-NMR method is based on the relaxation times of hydrogen nuclei in different phases of the sample. It is most often used to measure lipid content in food and seeds, but recent studies have applied it to microalgae. This method is non-destructive, with high accuracy and reproducibility. HPLC and TLC are also powerful methods of lipid analysis (Laurens and Wolfrum, 2011) but require considerable sample preparation time prior to analysis (Han et al., 2011). GC is a desirable method to measure and quantify lipids because it provides detailed information on fatty acid composition (Kramer et al., 2008; Mossoba and Kramer, 2009), but it is time-consuming as well (Mossoba et al., 2012).

FTIR spectroscopy measures the absorption of infrared (IR) energy at spectral locations indicative of the chemical bonds in molecules. The method is tolerant to small amounts of biomass in samples and requires relatively little sample preparation (Laurens and Wolfrum, 2011). FTIR spectroscopy can provide measurements with accuracy comparable to the gravimetric method, while being faster and more readily automated (Han et al., 2011).

FTIR transmission spectroscopy in the mid-infrared (MIR) range has been used for fast, reliable, high-sensitivity measurements of algae concentration and lipid content (Dean et al., 2010; Laurens and Wolfrum, 2011; Wagner et al., 2010). Lipids in algae contain different chemical groups like the methylene group (CH_2) and the carbonyl group (C=O) that absorb light in the MIR region due to their stretching and bending characteristics. According to Smith (1999), the absorption of energy at which vibrational stretching occurs for the CH_2 group is approximately

3000 cm^{-1}, and for the C=O groups is between 1800 cm^{-1} and 1600 cm^{-1}. Laurens and Wolfrum (2011) developed multivariate calibration models to predict the levels of neutral and polar lipids in spiked microalgae samples based on NIR and FTIR spectroscopy. They identified three distinct absorption bands for characterizing lipid content. Two of the bands are in the range 3025 to 2954 cm^{-1} for characterizing $–CH_3$ and $–CH_2$ groups, and one band for characterizing the ester C=O group is in the range 1746 to 1654 cm^{-1}. The models produced with FTIR spectroscopy provided good estimates of lipid contents and had R^2 values above 0.90.

The ultimate goal of this research was to develop a real-time lipid-content sensing system for algae in cultivation systems. To that end, the objective of this particular study was to test MIR-FTIR transmission spectroscopy as a method to estimate lipid content in a particular type of algae, *Nannochloropsis salina*, which has undergone extensive research as a potential biofuel source.

2 Methods

2.1 Algal solutions

Five bulk samples of *N. salina* at various lipid contents were provided by the Texas A&M AgriLife Research algae test facility in Pecos, Texas. The samples were produced and harvested in a way that assured samples of significantly different lipid contents. A gas chromatography/mass spectrometry with flame ionization detector instrument (GCMS–FID) (models 6890N and 5975B, Agilent Technologies, Santa Clara, CA, USA) was used to measure the fatty acid methyl ester (FAME) components from C_{14} to C_{24}. Neutral lipid was then calculated as the sum of all component FAMEs. The lipid content in the five samples ranged from 26.7 mg g^{-1} to 158.3 mg g^{-1}. The bulk samples were received from the Pecos facility as refrigerated algal solutions and were kept refrigerated at 4℃ throughout the study.

2.2 Algae-KBr pellets

Water is known to absorb energy strongly over certain portions of the IR spectrum and thus interfere with IR measurements (Pejcic et al., 2009). Therefore, sub-samples of the algae were dried thoroughly through centrifugation and oven drying prior to spectroscopic measurements. The sub-samples were placed in vials, inserted into the centrifuge (model Z 300 Hermle, Labnet International Inc., Edison, NJ, USA), and run at 2300 rpm for 20 minutes. They were then oven-dried for 12 hours at 40℃.

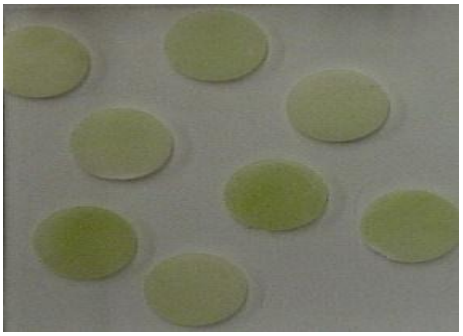

Figure 1 Algae-KBr pellets

Another source of measurement interference in transmission spectroscopy comes from opaqueness, which can prevent energy from reaching the detector. Therefore, one part of each dried algae sub-sample was mixed with 150 parts of the IR-transparent potassium bromide (KBr). The mixture was then ground with a mortar and pestle to produce a fine, even powder blend. Pellets were formed by compressing 135 mg of the mixed powder at high pressure (5 to 6 bar) for 5 minutes with a benchtop manual hydraulic press (Carver Inc., Wabash, IN, USA) with a 13-mm diameter die, producing a pellet of about 1 mm thickness (Figure 1). Pellet quality was checked by physical observation for cloudiness and breakage to ensure reliable measurements. A high degree of cloudiness indicated insufficient drying or moisture contamination from the ambient atmosphere, and breakage indicated poor mixing and grinding of

the samples. Pellets of low quality were discarded and replaced with high quality ones.

2.3 Transmission-FTIR spectroscopy

The transmission spectrum of each algae-KBr pellet was acquired from 4400 to 400 cm^{-1} with an FTIR spectrometer (Model 6700, Thermo-Nicolet, Madison, WI, USA). Spectra were analyzed with OMNIC software (Thermo Scientific, Madison, WI, USA) in an effort to precisely identify diagnostic wavenumbers. The absorbance at each diagnostic wavenumber was calculated by subtracting the local spectral baseline from the peak absorbance (Figure 2).

2.4 Identifying the absorption bands & calculation of absorbance

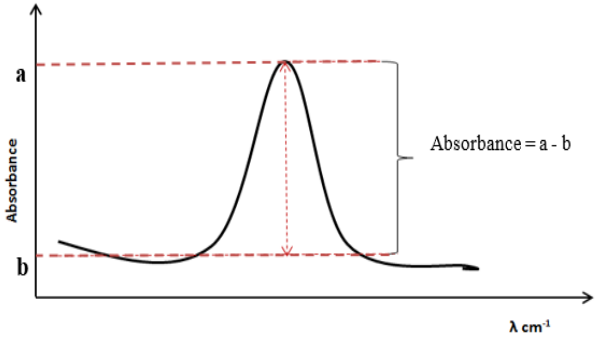

Figure 2 Calculation of absorption at the diagnostic band

2.5 Statistical analysis

Simple linear regression analyses were performed with JMP (SAS Institute, Cary, NC, USA) statistical software. The absorbance values at each diagnostic absorption band were compared to the neutral lipid content for all algae samples. Statistical parameters of primary interest were the coefficient of determination (R^2) as a metric of the data's explanatory power and the root mean square error (RMSE) as a metric of the precision of lipid-content estimates.

3 Results

3.1 Absorption spectra

A graph of spectra for two algae-KBr pellets of different lipid contents (39.5 and 94.7 $mg\ g^{-1}$) illustrates the common spectral curve shape and common diagnostic absorbance bands (Figure 3). All spectra for the various samples had the same unique features and generally conformed to the findings of Laurens and Wolfrum (2011), that energy absorption by algae lipids occurs at three diagnostic bands, in the current case defined as 2920, 2855, and 1742 cm^{-1}. The highest absorption intensity occurred at the diagnostic band 2920 cm^{-1}, followed by 2855 and 1742 cm^{-1}. The spectra also show some absorption bands related to other biochemical groups in the algae samples including protein at 1545 cm^{-1} and carbohydrates at 1150 cm^{-1}.

3.2 Regression analysis

The regression analyses produced models for correlating lipid content with absorbance measurements at each diagnostic band. The analysis at 2920 cm^{-1} had an R^2 value of 0.92 and an RMSE value of 10.6 mg g^{-1} (0.52%). The analysis at 2855 cm^{-1} had an R^2 value of 0.89 and an RMSE value of 11.8 mg g^{-1} (0.82%). Finally, the analysis at 1742 cm^{-1} had an R^2 value of 0.58 and an RMSE value of 23.4 mg g^{-1} (1.56%). Clearly, the diagnostic bands at 2920 and 2855 cm^{-1} produced high R^2 values and low RMSE values, indicating that these bands can both be used to estimate lipid content in *N. salina* (Figures 4 and 5). The band at 1742 cm^{-1} had a lower R^2 value and a higher RMSE value than the other bands, indicating relatively poor estimation performance (Figure 6). Based on the literature, the bands at 2920 and 2855 cm^{-1} are related to the presence of methylene groups in the samples, while the band at 1742 cm^{-1} is related to the presence of the carbonyl group, and they represent the absorbance of light due to fundamental vibrational transitions.

4 Future research

As previously stated, the ultimate goal of this research was to develop a real-time sensor for measuring algae lipids. In this study, transmission

FTIR showed promising results and partially overcame some of the disadvantages associated with conventional techniques, mainly the excessive time required for sample preparation. However, the technique still requires considerable sample preparation time to dry algae samples and produce the algae-KBr pellets. Thus, while diagnostic bands have been confirmed with transmission FTIR, attenuated total reflection-infrared (ATR-IR) may be more amenable to real-time sensing because it may allow samples to be measured with even further-reduced sample preparation time. According to Schuttlefield and Grassian (2008), transmission FTIR and ATR-IR techniques produce similar absorption spectra, so the results of this study provide some confidence that ATR-IR may also produce good results.

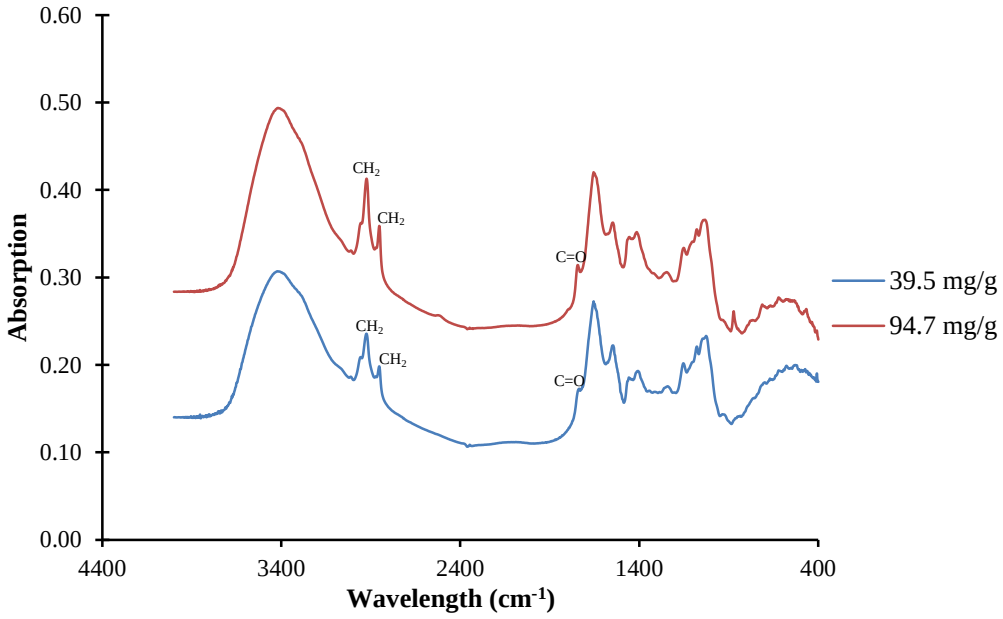

Figure 3 Absorption spectra graph of two algae-KBr pellets with different lipid content

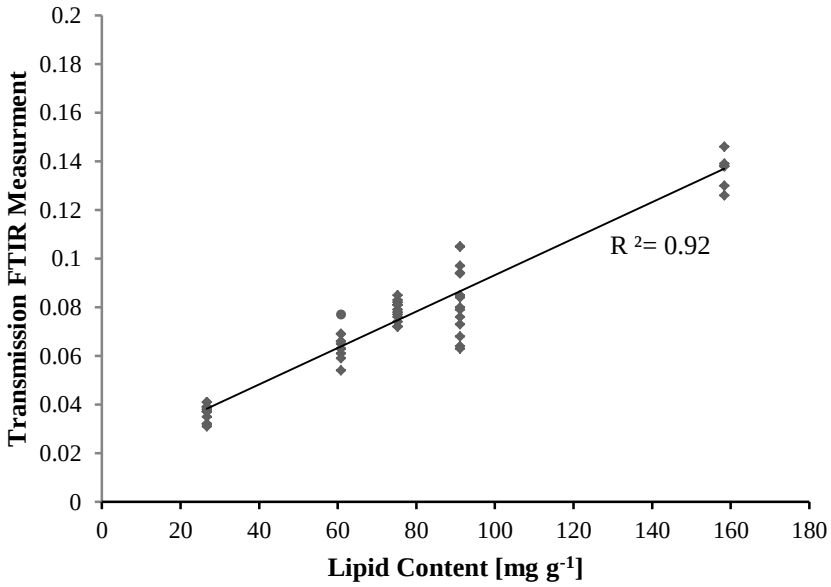

Figure 4 Actual lipid content versus transmission FTIR measurements (absorbance) for 2920 cm^{-1} waveband

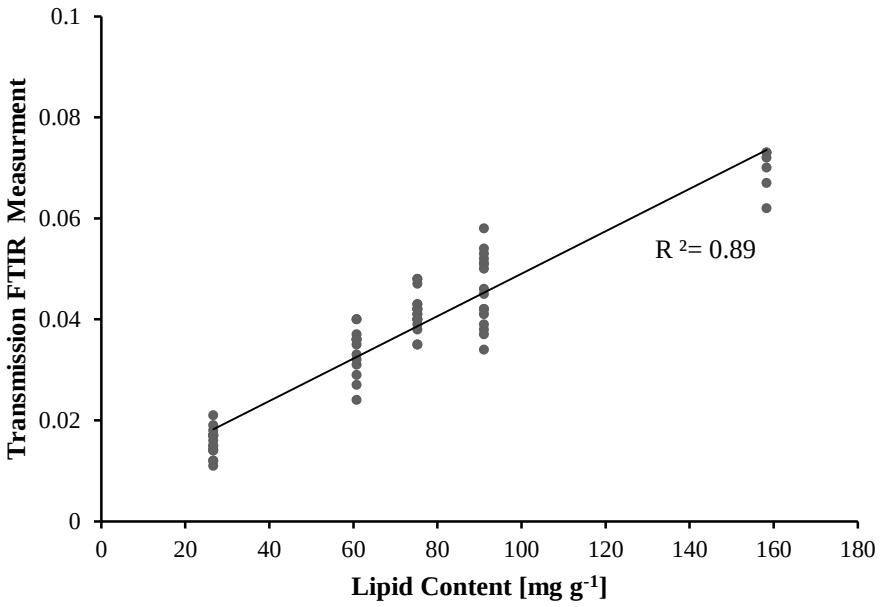

Figure 5 Actual lipid content versus transmission FTIR measurements (absorbance) for 2855 cm^{-1} waveband

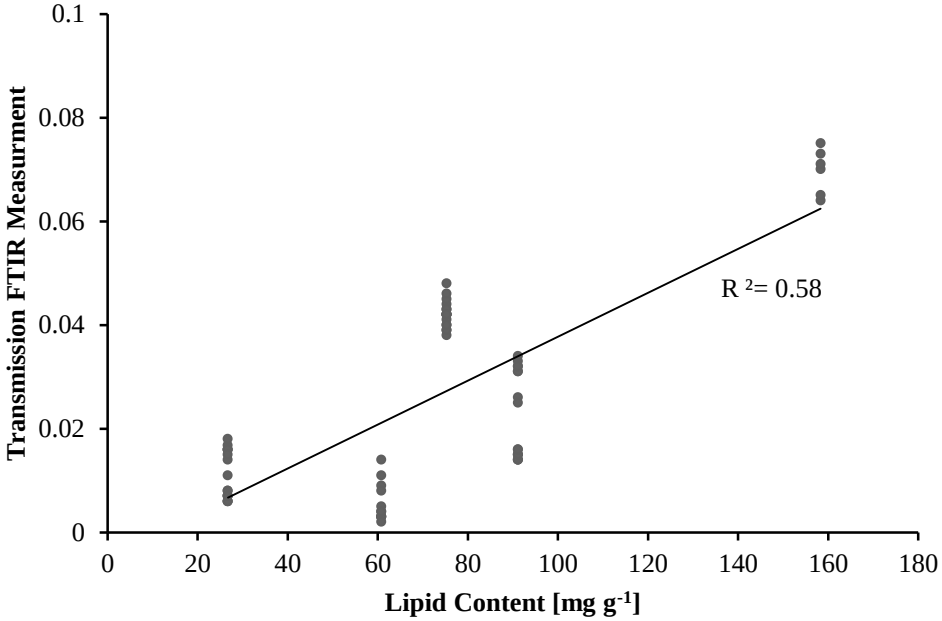

Figure 6 Actual lipid content versus transmission FTIR measurements (absorbance) for 1742 cm^{-1} waveband

5 Conclusions

Three diagnostic absorption bands (2920, 2855, and 1742 cm^{-1}) were identified as being useful in characterizing neutral lipids in algae (*N. salina*) with transmission FTIR spectroscopy of algae-KB pellets. The bands identified were in keeping with previous research. While this technique provided estimates of neutral lipids at each of the absorption bands, the band at 2920 cm^{-1} exhibited the strongest correlation and provided the best estimate (R^2 = 0.92, RMSE = 10.6 mg g^{-1}, or 0.52%). Further research is required to develop real-time lipid-sensing techniques for implementation in a process-control system.

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