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CHARACTERISATION OF POLYSACHARIDES AND LIPIDS FROM SELECTED GREEN ALGAE SPECIES BY FTIR-ATR SPECTROSCOPY

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Abstract

Fourier transform infrared (FTIR) spectroscopy was used in this study to identify and determine spectral features of Chromochloris zofingiensis (Dönz) Fucíková et L.A.Lewis (SAG 211-14, Gottingen, Germany), Acutodesmus obliguus (Turpin) Hegewald (SAG 276-1, Gottingen, Germany) and Chlorella sorokiniana (K. Brandt) Pröschold et Darienko (SAG 211-40c, Gottingen, Germany). Polysaccharides and lipids from these three algae species were determined using Fourier Transformed Infrared Spectroscopy (FTIR) with ATR accessory with diamante crystal in spectral range from 400 – 4000 cm^{-1} and resolution 4.

Key words

polysaccharides, lipids, algae, FTIR-ATR, Chromochloris zofingiensis, Acutodesmus obliguus, Chlorella sorokiniana

INTRODUCTION

It is now widely accepted that the end of cheap fossil oil era is already here and that the prices of crude oil will further increase in the years to come. This, along with the desire to control the greenhouse gases, urges the society to develop alternative sources for energy carriers and materials. Biomass is seen to be an environmentally safe and economically feasible alternative to fossil fuels. Common crops such as sunflower, palm, rapeseed, soybean, coconut, etc. are considered as first generation biodiesel feedstocks for biodiesel production. However, use of these feedstocks has faced problems as they disturb the overall worldwide balance of food reserves and safety (1).

The second generation of biofuels crops comprises lignocellulose biomasses such as cereal straw, sugar cane bagasse, forest residues; and organic components of municipal solid wastes (2). Of the biochemical and thermochemical options, pyrolysis is reportedly one of the few economically viable methods. Pyrolysis, or gasification, is the thermo-chemical breakdown of lignocellulose feedstock in the absence of oxygen, resulting in the production of chemical building blocks: hydrogen, carbon monoxide, CO_2, and other gases, which can be assembled into final products: alcohols (methanol and ethanol). With respect to the second generation biofuels, it is important to also consider the consequences of feedstocks derived from the agricultural sources produced on farmed or marginal land, the costs of fertilisation, and the consequences of land-use change. Specific impacts of the second generation biofuels may include changes in the carbon stocks of modified ecosystems caused by the removal of residues from agricultural regions, which represents a sink for both carbon and macronutrients (N, P, K). This may result in increased soil erosion, and the emission of nitrous oxide (a potent GHG) produced by soil bacteria. It is also important to determine whether growing biofuel crops poses local threats to biodiversity, or to water and nutrient cycling (3, 4).

The third generation biofuel feedstocks are generally regarded as those derived from eukaryotic microalgae and prokaryotic cyano-bacteria. These unicellular microorganisms provide an efficient link between carbohydrate assimilation and biofuel feedstock synthesis – typically fatty acids, but also starch – combined with productivities that are several fold greater than competing first and second generation biofuel technologies The use of unicellular microalgae and cyanobacteria avoids the issues that limit the sustainability of the first and second generation technologies. Microalgae and cyanobacteria, like second generation feedstocks, avoid competition for agriculture resources, arable land and potable water. In addition, the use of wastewater has the potential to provide essential nutrients required for growth, including N, P and carbon (specific to heterotrophic microalgae) [4]. Microalgae biomass (MBA) is potential feedstock for biofuel production because, they can produce quantities of polysaccharides (sugar) and triacylglycerides (lipid). These are the raw materials for bioethanol or biodiesel fuels (3).

FTIR spectroscopy is a relatively new technique used to measure cell composition of microalgae. FTIR spectra depict the macromolecular composition of the biomass on the basis of the infrared absorption of functional groups. Thus, FTIR spectroscopy allows the detection of changes in the relative abundance of organic pool such as carbohydrate, lipid and protein. This approach has been used in several studies on monitoring the changes in the macromolecular composition of microalgae induced by nutrient stress. FTIR spectra have been used as a fingerprint of the biochemical composition of the algal cell (5, 6).

MATERIALS AND METHODOLOGY OF EXPERIMENT

Freshwater algae *Chromochloris zofingiensis* (Dönz) Fucíková et L.A.Lewis (SAG 211-14, Gottingen, Germany), *Acutodesmus obliguus* (Turpin) Hegewald (SAG 276-1, Gottingen, Germany) and *Chlorella sorokiniana* (K. Brandt) Pröschold et Darienko (SAG 211-40c, Gottingen, Germany) were cultivated in ES (Erddekokt and Salze) basal medium containing soil extract, salt, and micronutrients, in a continuous airlift system and light-dark regime 14/8 hours, at 23 – 25 °C.

For FTIR analysis, 100 mL of algae samples were spread over a clean glass plate for air drying. The dried biomass was further broken into powder. Identification of polysaccharides and lipids in algae was performed using an Infrared Fourier Transform Spectroscopy with ATR technique - Attenuated Total Reflectance (Varian 660 Dual MidIR MCT/ TGS Bundle).

Samples were directly applied to a diamante crystal of ATR and the resulting spectra of them were corrected for background air absorbance. The spectra were recorded using a Varian Resolutions Pro and samples of starches were measured in the region 4000 - 400 cm^{-1}; each spectrum was measured 256 times, at resolution 4. To minimize differences due to baseline shifts, the spectra were baseline corrected and ATR-corrected.

RESULTS AND DISCUSSION

The FTIR spectrum reflects the distribution of the macromolecular pools. There are three main regions that relate to macromolecular pools, according to (7) the lipid band (around 1740 cm^{-1}), the amide I and amide II bands representing proteins (around 1660 and around 1540 cm^{-1}) and the carbohydrate region (1200 – 900 cm^{-1}).

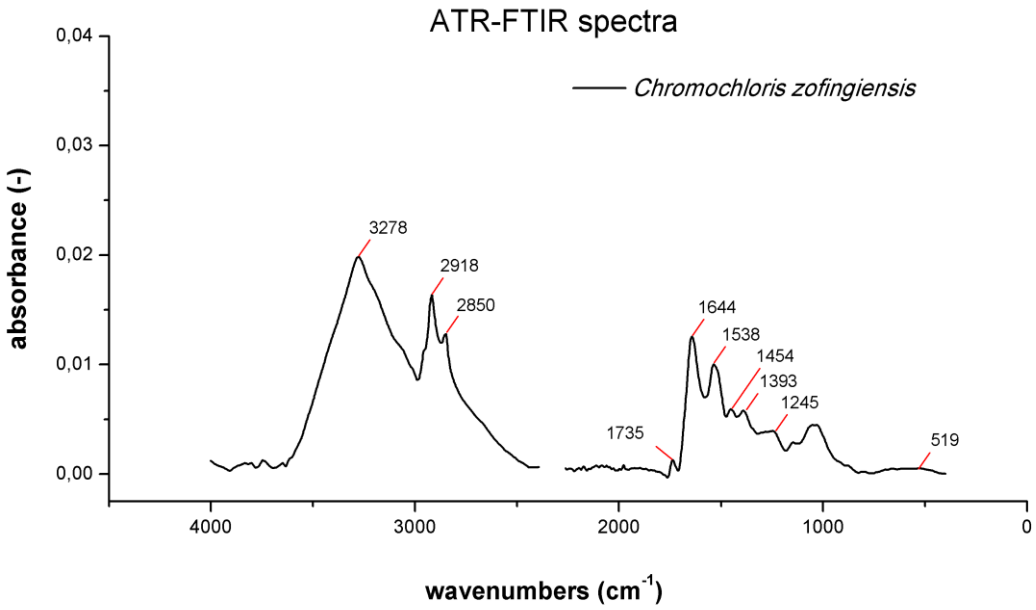

Fig. 1 *Infrared spectrum of alga Chromochloris zoofingiensis*

In Figure 1, each peak was assigned to a functional group. Protein spectra were characterized by two strong features at 1644 cm^{-1} and 1538 cm^{-1}. These bands were due primarily, to C=O stretching vibration and a combination of N-H bending and C-N stretching vibrations in amide complexes, respectively. Lipid spectra were characterized by two sets of strong vibrations, the C-H at 2850 cm^{-1} to 2918 cm^{-1}, and the C=O mode of the side chain from ester carbonyl group at 1735 cm^{-1}. In the spectrum, the region of CO_2 from the atmosphere is not presented.

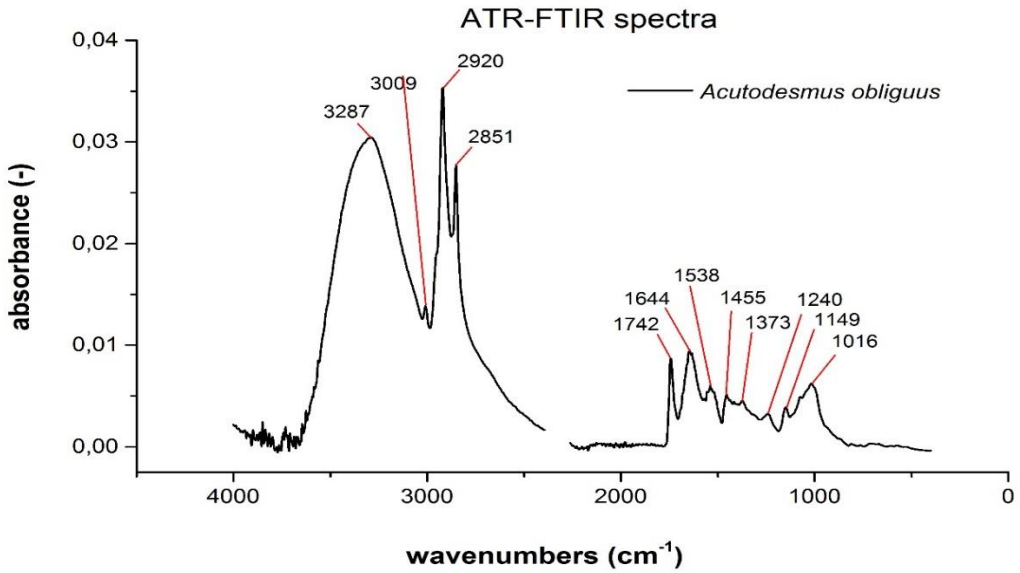

Fig. 2 *Infrared spectrum of alga Acutodesmus obliguus*

In Figure 2, protein spectra were characterized by strong peak at 1644 cm^{-1}, while amide spectra were presented by the peak at 1538 cm^{-1}. These bands were due primarily, to C=O stretching vibration and a combination of N-H bending and C-N stretching vibrations in amide complexes, respectively. Lipid spectra were characterized by two sets of strong vibrations, the C-H at 2920 cm^{-1} and the C=O mode of the side chain from ester carbonyl group at 1742 cm^{-1}, carbohydrate absorption bands due to C-O-C of polysaccharides at 1149 cm^{-1}, 1016 cm^{-1}, respectively (8). In the spectrum, the region of CO_2 from the atmosphere is not presented.

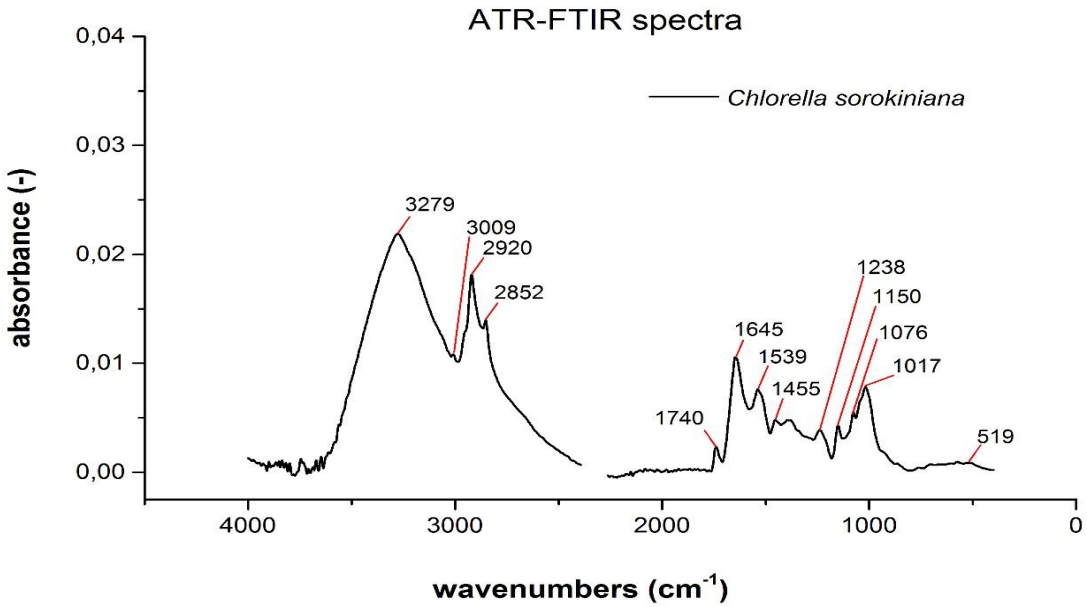

Fig. 3 *Infrared spectrum of alga Chlorella sorokiniana*

In Figure 3, intense absorbance between 2852 and 3009 cm^{-1} indicates the C-H stretching vibration of CH_3 and CH_2. The intense peak near the 1740 cm^{-1} region is ascribed to the C-O vibration developed in the presence of ketones, aldehydes, esters and carboxylic acids. The 1600–1500 cm^{-1} peak represents the amide-II band due to N-H bending. One of the major

monosaccharide in *Clorella sorokiniana* is glucosamine, an amino sugar, and therefore the presence of the amide bands might represent this amino sugar. The weak peaks in the 1238 – 1450 cm^{-1} region are due to the presence of C-O bending vibrations followed by C-H bending vibrations, further indicating the presence of a minor amount of esters (9, 10). In the spectrum the region of CO_2 from the atmosphere is not presented.

ASSIGNMENT OF BANDS FOUND IN FTIR SPECTRA OF *Cromochloris*. *zofingiensis, Acutodesmus obliguus*; AND *Chlorella sorokiniana* (5, 6, 9)

Table 1

Main peak (cm^{-1})			**Typical band assignment from the literature**	**Wavenumber range (cm^{-1})**
Chromochloris zofingiensis	***Acutodesmus obliguus***	***Chlorella sorokiniana***		
3278	3287	3279	Water *v*(O-H) stretching Protein *v*(N-H) stretching	3029-3639
2918 2850	3009 2920 2851	3009 2920 2852	Lipid – carbohydrate Mainly *v*as(CH2) and *v*s(CH2) stretching	2809-3012
1735	1742	1740	Cellulose–Fatty Acids *v*(C=O) stretching of esters	1763-1712
1644	1644	1645	Protein amide I band Mainly *v*(C=O) stretching	1583-1709
1538 1454	1538	1539	Protein amide II band mainly *δ*(N-H) bending and *v*(C-N) stretching	1481-1585
	1455	1455	Protein *δ*as(CH2) and *δ*as(CH3) bending of methyl, Lipid *δ*as(CH2) bending of methyl	1425-1477
1393	1373		Protein *δ*s(CH2) and *δ*s(CH3) bending of methyl Carboxylic Acid *v*s(C-O) of COO- groups of carboxylates Lipid *δ*s(N(CH3)3) bending of methyl	1357-1423
	1240	1238	Nucleic Acid (other phosphate-containing compounds) *v*as(>P=O) stretching of phosphodiesters	1191-1356
	1149	1150	Carbohydrate *v*(C-O-C) of Polysaccharides	1134-1174
		1076	Carbohydrate *v*(C-O-C) of polysaccharides Nucleic Acid (and other phosphate-containing compounds) *v*s(>P=O) stretching of phosphodiesters	1072-1099
	1016	1017	Carbohydrate *v*(C-O-C) of polysaccharides	980-1072

CONCLUSION

Macromolecular compositions of three algal species were determined with FTIR-ATR spectroscopy method. The aim of this paper was to prove that FTIR-ATR method is a good tool

for cheap, rapid and easy quantitative characterisation of polysaccharides in algae. Another advantages of his method is the use of small amounts of biomass material. Simultaneously, it is a good method for studying diversity under different types of cultivation conditions, for example light or nutrient concentration of the growing medium.

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