BIOACCUMULATION STUDY OF ACRYLATE MONOMERS IN ALGAE (*CHLORELLA KESSLERI*) BY PY-GC AND PY-GC/MS

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Abstract

Acrylate monomers methylmethacrylate (MMA) and cyclohexylmethacrylate (CHMA) bioaccumulation has been determined in aquatic organism, algae (*Chlorella kessleri*). Algae were collected in amount of 0,4 mg and directly injected to the pyrolytical cell. In algae bodies accumulated monomers were analysed by pyrolysis gas chromatography (Py-GC) and pyrolysis gas chromatography coupled with mass spectrometry (Py-GC/MS). Traces of the accumulated monomers in algae body can be determined after 1-, 2 -, 3-weeks of incubation. Maximum content of MMA was determined after 3-week of experiment, contrariwise in the case of CHMA after 2-week exposition. Relationship with pyrolysis temperature has also been studied.

Keywords: Pyrolysis gas chromatography (Py-GC), Pyrolysis gas chromatography-mass spectrometry (Py-GC/MS), MMA, CHMA, Algae (*Chlorella kessleri*), bioaccumulation

1. INTRODUCTION

Pyrolysis gas chromatography has been applied to the study of biological materials for more than two decades [1]. Much of the work has been done in an effort to identify and differentiate microorganism [2-3], such as the work by Smith et al. [4] who found a chemical marker for distinguishing Group A and Group B streptococci. Many biological materials including biomass [5], enzymes [6], steroids [7], have been assayed using Py-GC. Recently a method for analysing small quantities of samples was developed, and is based on either pyrolysis-GC (Py-GC) or Py-GC/MS [8]. *Russell* [9] used Py-MS for identification and systematisation of brown algae. *Cejka* [10] analysed by Py-GC/MS fossil organic matter from sediments, which consists of different type of algae. *Derrene* [11] used flash pyrolysis with GC/MS to characterise intractable organic molecules in algae.

Kruge [12] by Py-GC/MS studied aquatic organic matter in the open water sediment samples and he identified nitrogen compounds in the pyrolyzate including pyrroles, pyridines and indoles. These compounds are characteristic pyrolysis products of proteins and degraded proteinaceous matter, in this case largely from marine algae and bacteria. *Ishida* [13] applied, the reactive Py-GC to the determination of the lipid contents and their fatty acid composition of every zooplankter individual at different algae concentration. Algae (*Chlorella kessleri*) present an organism, which are belonging to the best natural detoxifier for heavy metals and other synthetics. *Chlorella* has been used extensively as a detoxifying agent for variety of heavy metals, insecticides and other toxins. The detoxification capability of *Chlorella* is due to its unique cell wall and the material associated with it. The cell walls of *Chlorella* have been shown to have three layers of which the thicker middle layer contains a polymerised carotene material. Laboratory studies showed that there were two active absorbing substances – sporopolenin (a naturally occurring carotene like polymer which is resistant to degradation) and the algae cell walls [14]. Most studies on chemical components in aquatic organisms have been made by extraction and transmethylation followed by using gas chromatography (GC) or liquid chromatography (LC) [15-17]. Such conventional methods, however, are not effective for analysis of small organisms such as phytoplankton (algae), because a large quantity is required for analysis. Furthermore, it is difficult to evaluate the biological effect of the components on an individual organism and the accumulation of specific compound, because biological changes in each organism are affected by the organism's life history, which includes exposure to many different environmental conditions [18]. Sometimes the results of the laboratory and field experiments are not directly comparable, zooplankton may accumulate more pollutants if incubated with phytoplankton such as algae [19].

In last years incoming elevated produces of acrylates in paint, textile and paper industry. Therefore is necessary to identify this type of compound (monomers, polymers) in waste waters. Methylmethacrylate is used preliminary to make variety of resins and plastics and is most often polymerized to polymethyl methacrylate, which is used to make acrylic sheets, acrylic moldings and extrusion powders. Cyclohexylmethacrylate present base material for coatings and adhesives [20].

This paper deals with determination of MMA and CHMA in algae (*Chlorella kessleri*) body. This algae strain has been growth in water solution containing with MMA and CHMA. Accumulation of these monomers was study by Py-GC after 1-, 2- and three weeks. Pure algae (*Chlorella kessleri*) and MMA, CHMA were investigated by Py-GC/MS.

2. EXPERIMENTAL

2.1 Chemicals and material

Algae strain (*Chlorella kessleri*) was purchased from Botanic Institute, Bratislava (Slovakia) and magnesium sulphate, di-potassium hydrogen phosphate, potassium nitrate, iron chloride were available from Lachema Brno (Czech Republic). Cyclohexylmethacrylate (CHMA) and methylmethacrylate (MMA) were purchased from Sigma-Aldrich GmbH (Germany) with p.a. purity. Special pelletiser syringe has been used for samples introduction into the pyrolyser for Py-GC/MS analysis. Balance (Mettler AE 240) from Mettle instrument, Switzerland was used.

2.2 Algae samples

The phytoplankton samples (*Ch.kessleri*) were cultivated in our laboratory in specific medium. Cultivation medium consists of: 1l distilled water and 10 ml 1% Potassium nitrate, 1 ml 1% Magnesium sulphate, 1 ml 1% di-Potassium hydrogen phosphate, 0,2ml 1% Iron chloride. The original solution was exposed to oxygen and light overnight under room temperature. The phytoplankton samples (*Ch. kessleri*) were exposed in a static renewed system for 1- week to 0,08 ppm of CHMA and 0,4 ppm of MMA solution. After 1-,2- and 3-week exposition the medium was centrifuged, filtered, washed with re-distilled water and then analyzed.

2.3 Pyrolysis-gas chromatography (Py-GC)

Coupled Py-GC system contained a microfurnace pyrolyzer (SGE, Pyrojector II, Australia) with GC 9000 Series (Fisons) gas chromatograph. Pyrolysis of treated sample was performed at 600, 700, 800, 850°C for monomers nonexposed algae. The algae samples after 1-,2-,3,-week incubation were inserted to the solid sampler and analysed at 850°C. Separation of pyrolytical products was achieved on a 30m Sol Gel Wax capillary column: inner diameter, 0,25 mm, film thickness-0,25μm (SGE, Australia). The column temperature was kept at 70°C for 2 minutes and programmed to 170°C at the increments of 10°C/min. The GC conditions were: injector at 150°C, flame ionization detector (FID) at 150°C, nitrogen as carrier gas (flow rate, 0,6 ml/min). Split injection mode has been established with 1/25 splitting ratio. Internal volume of liner in pyrojector was 0,43cm^3 and the nitrogen gas pressure was set to 165kPa. Flash pyrolysis has been applied.

2.4 Pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS)

The pure algae sample was injected into the pyrolyzer and pyrolysed at 500, 600, 700, 800, 850°C. Py-GC/MS analysis was made by using a combination of a model microfurnace pyrolyzer (SGE, Australia) and GC/MS (Saturn Varian 2100 T). The GC was operated at an injector temperature of 150°C, helium as a carrier gas 1 ml/min, and a DB-5 capillary column (30m x 0,25mm ID x 0,25 μm thickness) was used. The column temperature was kept at 70°C for 2 minutes and programmed to 170°C at the increments of 10°C/min. The mass spectra of compounds were measured at 70eV. Mass spectra library (NIST 98) was used at spectra interpretation.

3. Results and discussion

3.1. Results from MMA, CHMA, algae analysis by Py-GC.

Nonexposed strain of *Chlorella kessleri* was analysed at different pyrolysis temperatures. The chromatograms showing pyrolysate composition changes at different temperatures are given at Fig.1 (a-d). At 600°C algae bodies decomposed completely; the most analytes from pyrolysate were obtained. These compounds have the same retention times as the analytes from monomers MMA and CHMA analysis and they can to be coeluted or lost in the background signal. We obtained much more simple pyrogram at 800°C-850°C. At these temperatures were both monomers well separated and detected. First four main peaks presented the fingerprints of monomers with retention time in the interval up to the 6 minutes.
This monomers fingerprint was used for the next identification in bioaccumulation study. From Py-GC/MS analyses of MMA and CHMA in algae body it was resulted that degradation of CHMA started at 600°C. At 700°C MMA and CHMA completely degraded and at 800°C they were observed molecules of naphthalene, acenaphtene, acenaphtylenes (Fig.2). These results were confirmed with MS spectra analysis. Optimal degradation temperature to get fingerprints for MMA and CHMA to avoid simultaneous interference of algae degradation products ranged from 750-850°C.

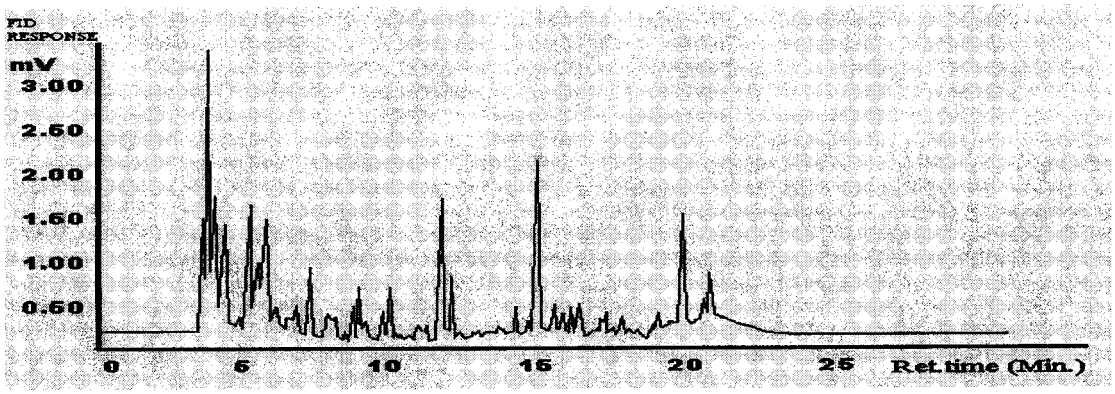

Fig. 1 (a) Pyrogram of Algae (*Chlorella kessleri*) obtained by Py-GC analysis
a = pyrolysis temperature 600°C

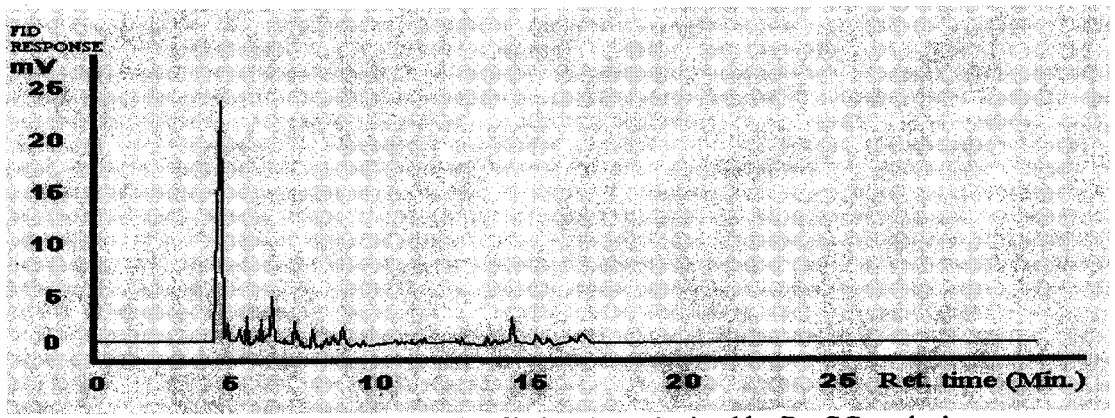

Fig. 1 (b) Pyrogram of Algae (*Chlorella kessleri*) obtained by Py-GC analysis
b = pyrolysis temperature 700°C

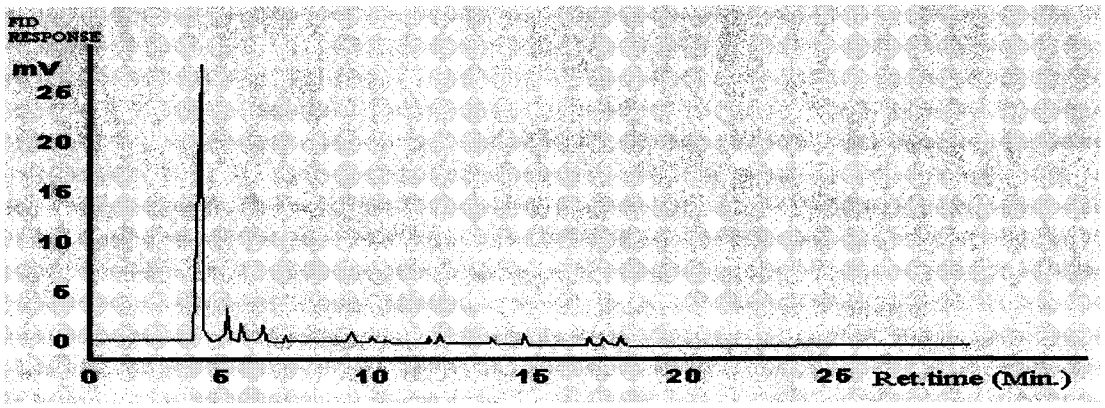

Fig. 1 (c) Pyrogram of Algae (*Chlorella kessleri*) obtained by Py-GC analysis
c = pyrolysis temperature 800°C

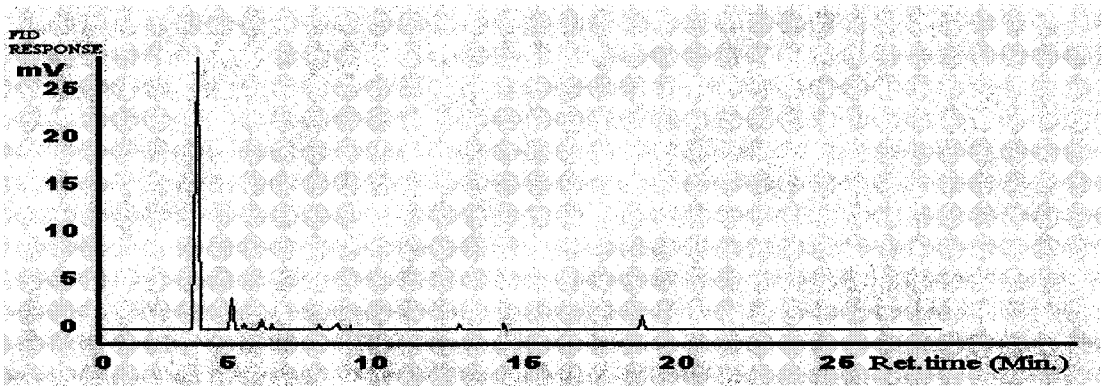

Fig. 1 (d) Pyrogram of Algae (*Chlorella kessleri*) obtained by Py-GC analysis
d = pyrolysis temperature 850°C

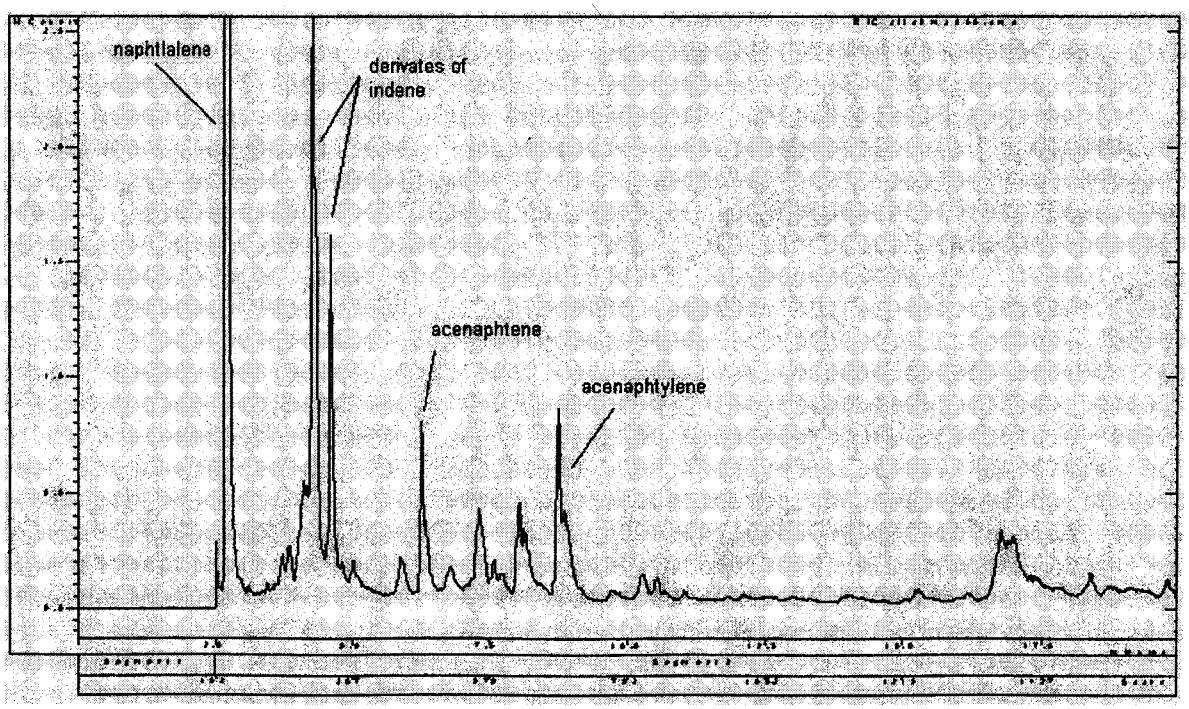

Fig. 2 Pyrogram of CHMA after Py-GC/MS analysis at 850°C

3.2. Results from algae strain Py-GC analysis after CHMA long-term exposition

Chlorella is a key-detoxifying agent used during the removal of heavy metals, pesticides and other toxins. The main aim of this work was find a bioaccumulation of toxic monomers in *Chlorella kessleri* by Py-GC analysis. The first step of this experiment was executed a toxicity test of measured monomers. Lethal concentration of CHMA for algae was 0,1 ppm. Presented monomer was added to algae samples under the amount, which presented the lethal concentration (CHMA 0,08 ppm). Analysis of prepared sample was executed at 850°C. CHMA accumulation was identified after 1-week incubation, however the highest amount of CHMA has been determined from calibration curve and maximum accumulated amount in algae body was 0,2 ppm after 2-week exposition. This amount was two times higher as than the acute toxicity of CHMA in *Chlorella* body. Pyrograms declaring CHMA content in algae body after 3 weeks incubation are given in the Figure 3. In the case of CHMA the highest intensity of peaks (which belong to degradation products of CHMA) and the number of pyrolytical products we observed after 2-week incubation. The highest amount of CHMA in *Chlorella* bodies was detected after 2-week exposition.

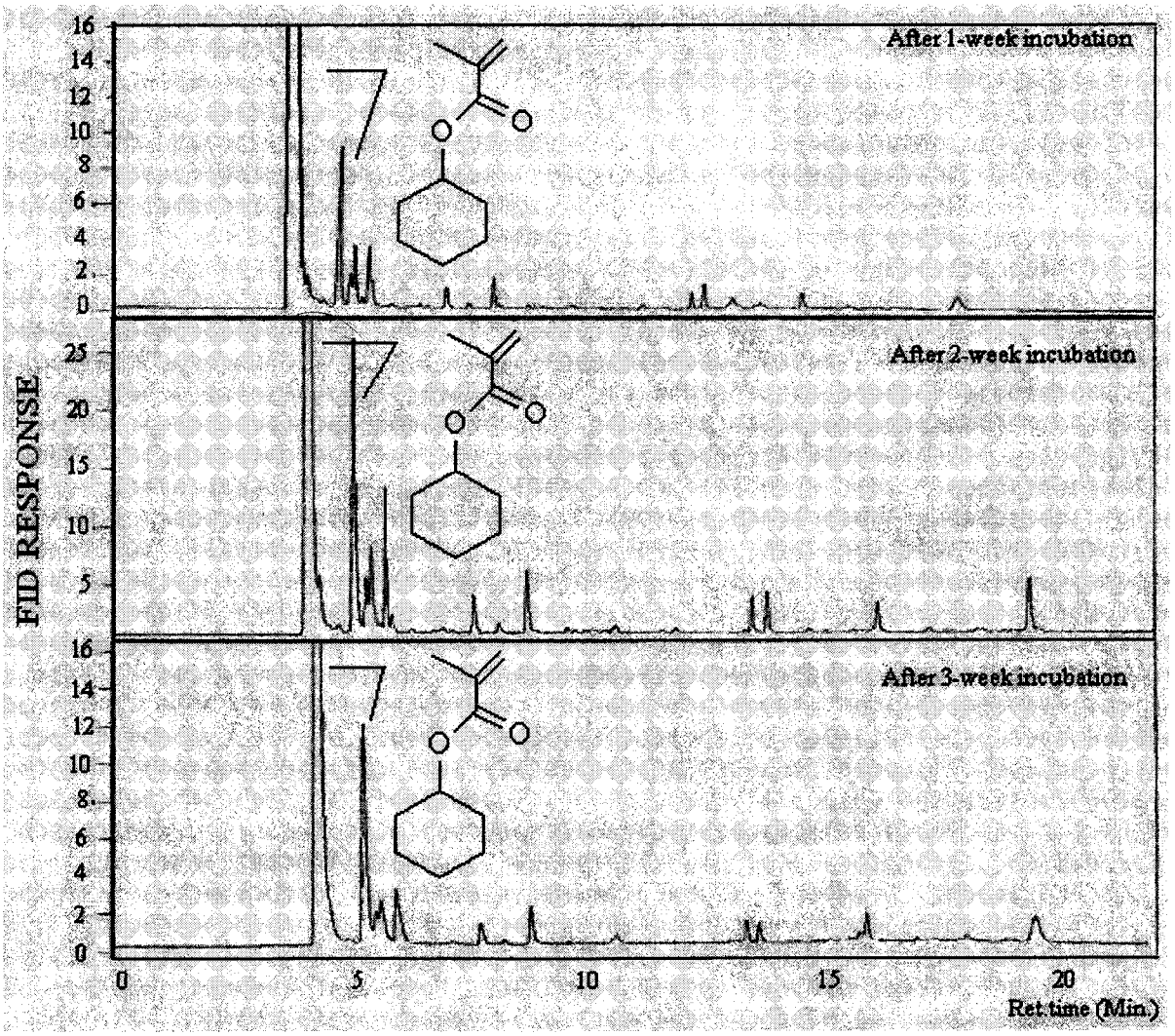

Fig. 3 Pyrograms of algae with CHMA after 1-, 2-, 3-weeks incubation

3.3. Results from algae after MMA long-term exposition

Fig. 4 shows the pyrograms of algae with MMA (0.4 ppm) after 1-,2-,3-week incubation. Although the observed accumulation of MMA was negligible during the 1st week of exposure, a significant change in the concentration was detected after 1-week exposure. During the first week exposure amount of pyrolysates and abundance of detected peaks was the same as the pyrolysates from original algae strain. A significant change we observed after 2- and 3-week exposure while the abundance of peaks and the amount of pyrolysates were gradually increased. After toxicity test of *Chlorella* by MMA (LC of MMA for *Chlorella* was 0,5 ppm) was added to original solution 0,4 ppm of MMA. The highest amount of MMA has been identified after third week exposition from calibration curve as in CHMA case and the maximum accumulated amount of MMA was 1,5 ppm. The highest amount of MMA was identified after 3-week exposition but this amount was three times higher as than the acute toxicity of MMA in algae body. According to a Finish report of long-term experiments by a model ecosystem in Baltic Sea, the bioaccumulation of polychlorinated biphenyls and metabolites from water ranged from 50 times for algae to 700 times for invertebrates and fish [18]. It's very hard to ensure similar conditions such as in water environment. A remaining question is how complex an experimental system must be given a realistic assessment of the effect of effluents in ecosystem. Although the results of the laboratory and field experiments are not comparable, for example zooplankton accumulated more toxic compounds if incubated with phytoplankton such as algae, in a model ecosystem [18].

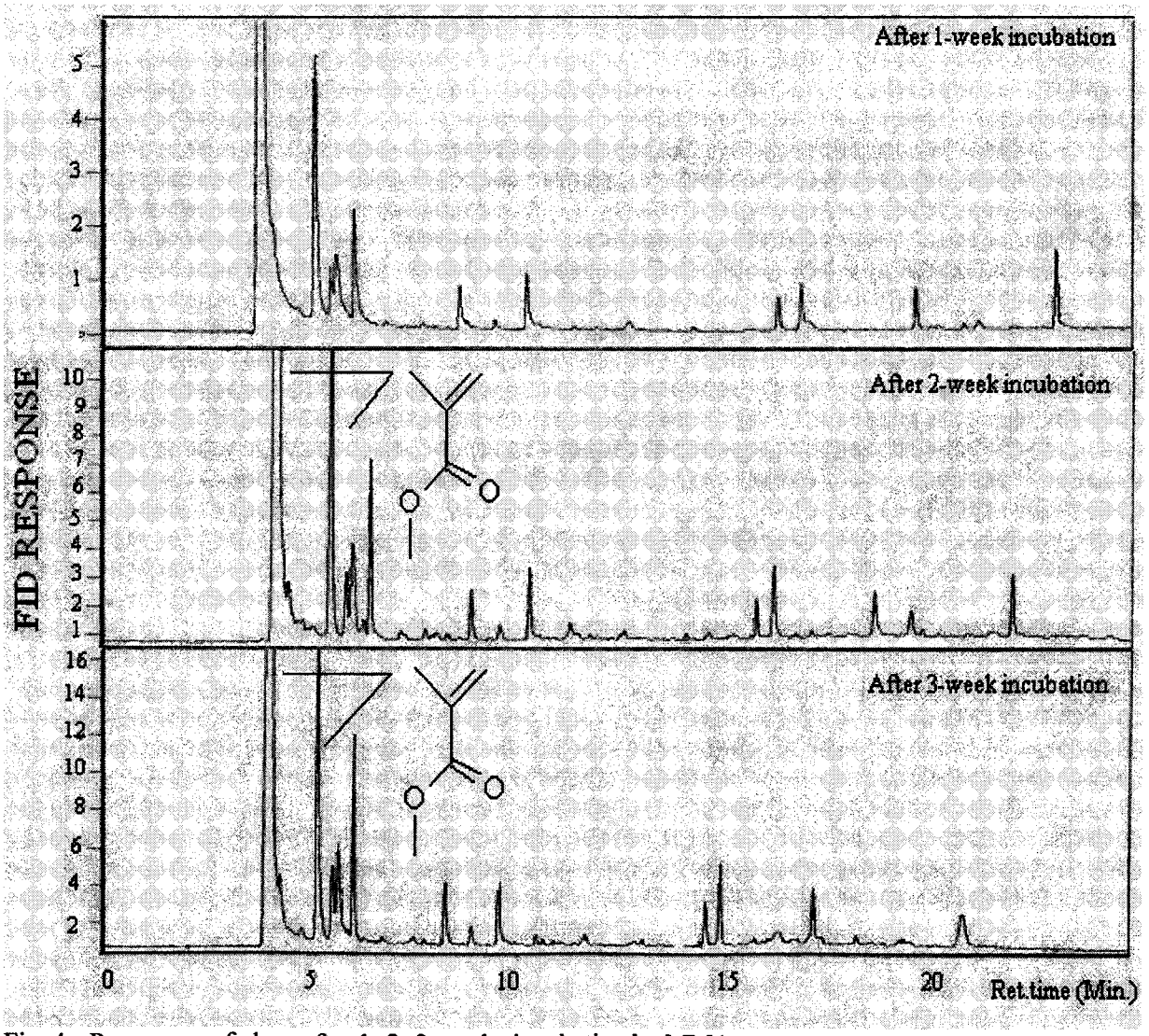

Fig. 4 Pyrograms of algae after 1-,2-,3-weeks incubation by MMA

4. CONCLUSION

Pyrolysis of pure algae (*Chlorella kessleri*) and bioaccumulation of monomers (MMA, CHMA) was investigated by Py-GC. By Py-GC/MS analysis were analyzed presented monomers. Polyaromatic hydrocarbons were identified after Py-GC/MS analysis of two monomers. Optimal degradation temperature to get fingerprints for MMA and CHMA to avoid simultaneous interference of algae degradation products ranged from 750-850°C.
Py-GC enables direct sampling of solid sample and also from this reason is appropriate on analysis of biological samples, which not require pre-treatment of sample; conventional GC or LC method require pre-extraction from larger amounts of organisms, and require a total analysis time of 2-3 days.
The highest accumulation of CHMA was observed after 2-week incubation, contrariwise MMA was not detected after 1-week exposition, but bioaccumulation of MMA was gradually elevated.

Acknowledgements

The authors wish thank for financial support by grant VEGA 1/0445/03.

REFERENCES

[1] T.P. Wampler, Journal of Chromatography A, 842 (1999) 207-220
[2] Goodacre R., Journal of analytical and applied Pyrolysis, 44(1997) 49-64
[3] Voorhees K.J. Journal of Anal. Appl. Pyrolysis, 40-41, (1997) 111-134
[4] C.C. Smith, S.L. Morgan, C.D. Parks, A. Fox, D.G. Pritchard, Anal. Chem. 59 (1987) 1410.
[5] Varhegy J., Journal of Analytical and Applied Pyrolysis, 42 (1997) 73-87
[6] N.D. Danielson, J.L. Glajch, L.B. Rogers, J. Chromatogr. Sci. 16 (1978) 455
[7] F.M. Menger, J.J. Hopkins, G.S. Cox, M.J. Malonez, F.L. Bazer, Anal. Chem. 50 (1978) 1135.
[8] T.Sekino, M. Nakanishi, Y. Ishida, S. Isomura, S.Tsuge, H. Ohtani, T.Kimoto, Freshwater Biology 38 (1997) 611
[9] G. Russell, Marine Biology (1995) 123, 153-157
[10] J.Cejka, Z.Sobalik, Collection of Czechoslovak Chemical Communication, (1997) 62, 364-374
[11] S.Derrene, C.Largeau, E.Cassadevall, Org. Geochemistry, 17, (1991) 597-602
[12] M.Kruge, G.Benoit, J. of Con.Abs., 5 (2000) 607
[13] Y.Ishida, H. Ohtani, S. Tsuge, T. Sekino, M. Nakanishi, T. Kimoto: Biol. Sciences in Space, Vol. 12 No.2 (1998):131-135
[14] www.chlorella-europe.com
[15] G.Kattner, H.S.G Fricke, J. Chromatography 361 (1986) 263
[16] T.Farkas, Comp. Biochem. Physiol. 64B (1979) 71.
[17] R.E.Lee, J.C. Nevenzel, G.-A.Paffenhofer, Marine Biology 9 (1971) 99.
[18] H. Araki, N. Tatarazako, K. Kishi, K. Kuroda, Jornal of Analytical and Applied Pyrolysis, 55 (2000) 69-80.
[19] K. Kuroda, A. Suzuki, M. Kato, K. Imai, Journal of Analytical and Applied Pyrolysis, 34 (1995) 1.
[20] J.S.Gift, Tox. Review of Methylmethacrylate, U.S.Protection Agency, Washington, 3-15 (1998)