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Biopigments, Obtaining and Properties

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Biopigments, Obtaining and Properties

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In this work we report some of the key parameters which determine pigment production in submerged biosynthesis using different nutrient sources like glucose, starch, rice, glutamic acid, with a low oxygen concentration in order to avoid the mycotoxin formation. The chemical composition of biopigment performed by fluorescent X-ray diffraction and atomic emission in coupled inductively plasma indicate a high concentration of C, N, H, and K and the presence of microelements like Co, Cu, Fe, Mn, Zn. The stability of bioproduct determined by electronic spectra in solid state and in different solvents (i.e., water, ethanol, diethyl ether and n-hexane) indicates the instability of bioproduct in the presence of light either in the solid state or in different solvents. Its fresh aqueous solution has strong antioxidant properties, comparable with those of E vitamin.

Keywords Antioxidant; ascomyces; biopigment properties

Introduction

In the last years the studies on the natural food colorants have intensified, with the main objective to replace the synthetic dyes. Microorganisms from *Ascomyces* family give pigments which are used for colouring the wine, red soybean cheese, [1,2] etc. Depending on nutrient sources from the system, yellow, orange and red pigments from *Ascomyces* can be obtained. In this work we report on the key parameters, which determine pigment production in submerged biosynthesis using different nutrient sources. In addition, the physico-chemical properties of biopigments are presented.

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Experimental

Two strains of Ascomyces species have been used in experiments, parental and modified strain. These are maintained on potato dextrose agar. One slants for each strain is used as seeds of inocculum, prepared in each case from different culture media (Table 1). Biosynthesis in the solid state culture media was performed in 500 mL Erlenmeyer flask. All experiments were conducted at 30°C, on the orbital shaker of 250 rpm. The UV-VIS spectra were recorded in the culture media or in solution by dilution with water or ethanol using a UV/VIS/NIR spectrophotometer type Perkin Elmer LAMBDA^{TM} 750 with Integrating Sphere. Studies regarding the stability of solid pigments synthesized in liquid or solid states were performed. Antioxidant capacity was calculated by fluorimetric method based on Oxygen Radical Absorbance. The chemical composition of biopigment was determined by fluorescent X-ray diffraction and atomic emission in coupled inductively plasma using a spectrometer type PW 4025 MiniPal and a spectrometer type ICP-AES Varian Liberty 110.

The infrared spectra were recorded using a FT-IR spectrophotometer type Spectrum GX Perkin Elmer with accessories: DRIFT (Diffuse Reflectance Infrared Fourier Transform) and ATR (Attenuated Total Reflectance).

Table 1. Culture media used in the pigment biosynthesis

Medium type		M.U. (g/L)	Medium type		M.U. (g/L)
Medium 1 (submerged biosynthesis)			Medium 5 (submerged biosynthesis)		
1	Dextrose	3	1	Na glutamate	5
2	$NaNO_3$	0.1	2	KH_2PO_4	5
3	$MgSO_4$	0.05	3	$MgSO_4 \cdot 7H_2O$	0.5
4	$FeSO_4$	0.01	4	$CaCl_2$	0.5
5	$ZnSO_4$	0.08	5	$FeSO_4 \cdot 7H_2O$)	0.5
6	$MnSO_4$	0.03	6	$ZnSO_4 \cdot 7H_2O$	0.01
7	Distilled water	up to 1L	7	$MnSO_4 \cdot H_2O$	0.03
Medium 2 submerged biosynthesis			8	ethanol	20
1	Starch	10	9	distilled water	up to 1L
2	Peptone	4	Medium 6 (submerged, modified Lin culture media)		
3	Glutamic acid	1			
4	Distilled water	up to 1L	1	dextrose	30
Medium 3 (semisynthetic culture media)			2	Na glutamate	3
1	rice powder	50	4	$MgSO_4 \cdot 7H_2O$	1
2	$NaNO_3$	5	5	$FeSO_4 \cdot 7H_2O$	14 mg/L
3	Distilled water	up to 1L	6	distilled water	up to 1L
Medium 4 (semisynthetic Lin culture media)			Medium 7 (solid state biosynthesis)		
1	rice powder	30	1	Rice powder	100 g
2	$NaNO_3$	1.5	2	distilled water	30 g
3	$MgSO_4 \cdot 7H_2O$	1			
4	KH_2PO_4	2.5			
5	Distilled water	up to 1L			

Results and Discussions

Productions of pigment in submerged media, using parental strain, reveal no pigment production after 120 h. In the presence of rice and starch, great quantities of red pigments were obtained when modified strain were used (optical density (OD) = 0.95 units is obtained after 96 h (Fig. 1)).

Modified strain produced also a large quantities of orange pigments (Fig. 2) in the presence of starch and rice corresponding to OD = 3 units and 1.5 units respectively, after 96 h. At the same time, yellow pigment present an optical density less than 0.5 units at 95 h (Fig. 3) but the quantities of these pigments increase slowly exhibiting an optical density equal to 2.5 units after 120 h. These results reveal the formation of orange and yellow pigments in the first step; after that all these compounds were transformed into red pigments. In fact it was obtained a mixture of these three dyes with very similar chemical structure (Fig. 4).

Large quantities of dextrose produce an amount of red pigment which correspond to OD = 0.85 units after 168 h; in this case the yellow pigment are formed in quantities corresponding to OD = 1.3 while the OD for the orange pigment is 0.5 units. After 120 h there is no production pigment in submerged media when parental strain was used.

In all cases, good results are obtained in the presence of rice, and starch culture media, when in the submerged media quantities of red pigments corresponding to OD = 0.95 units after 96 h are formed.

Experiments performed in liquid or solid-state media reveal an important efficiency of the last one on pigment production: for 100 g rice, 20.83 g pigment are obtained. The materials have three components: yellow, orange and red pigment, but their solubility in common solvent like water and alcohol is very small. For this reason, pigments obtained in solid media were studied in order to establish time stability. Experiments performed in solid state, at room temperature and light reveal a slowly decomposition of pigment in 30 day; after this period the dyes seems to rest stable (Fig. 5).

In ethanol media (red, orange, and yellow pigments extraction), biomaterial are decomposed in aprox 20 days (Fig. 6).

In acetone (orange and yellow pigments extraction) (Fig. 7) and N hexane (yellow pigments extraction) the decomposition takes place in 23–25 days (Fig. 8).

In water biopigments are strongly decomposed; but in the fresh solution, the biopigment mixture reveals a powerful antioxidant capacity (26.196×1000 T.E./mL) in comparison with E vitamin (Fig. 9).

Infrared spectrum (Fig. 10) shown the polysaccharidic fingerprint in the range 700–900 cm^{-1}; the carbonyl region is presented at 1264.65 cm^{-1}, 1374.59 cm^{-1} and the peak at 1457 cm^{-1} correspond to CH_3 group. The absorption of carbonyl, amide I and amide II at 1538.54 cm^{-1}, 1628.55 cm^{-1}, and 1715.47 cm^{-1} demonstrate the presence of yellow, orange and red pigment in the biomaterial (yellow and orange pigment have C=O group in the molecule; red pigment has a NH group in molecule). The peaks at 2927.57 cm^{-1} and 3277.07 cm^{-1} reveal the presence of C-H bond, which comes from fatty acids of the mixture.

Qualitative analysis performed in the first step by fluorescent X-ray (Fig. 11) reveals the presence of K, Ca, S, Al, P, Co, Cu, Fe, Mn, Zn in the biomaterials.

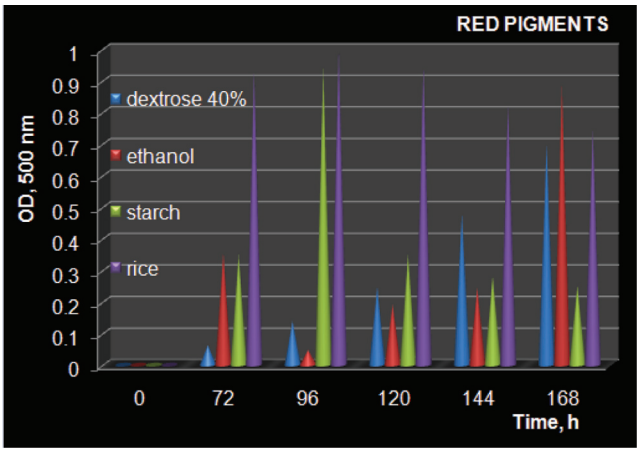

Figure 1. Influence of different submerged culture media on red pigment production.

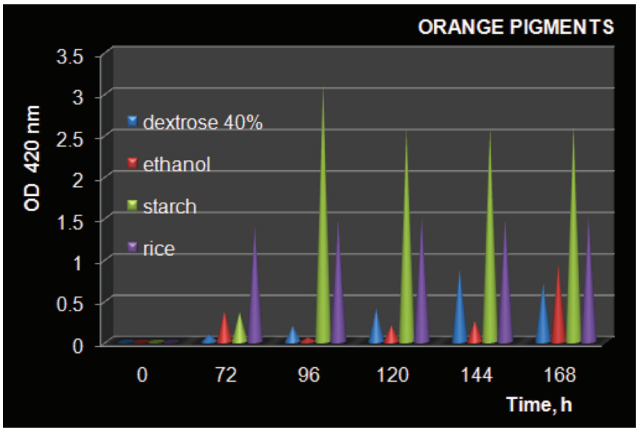

Figure 2. Influence of different submerged culture media on orange pigment production.

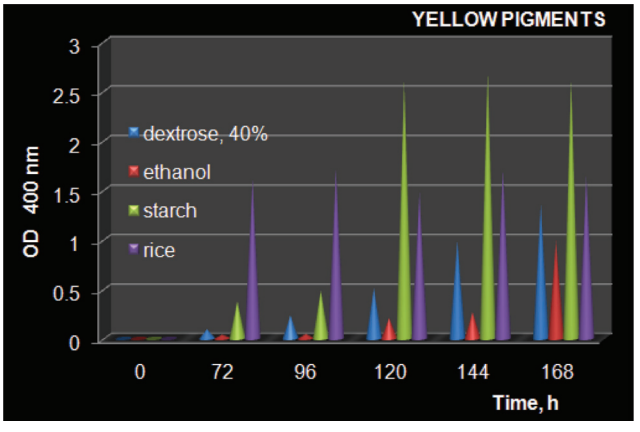

Figure 3. Influence of different submerged culture media on yellow pigment production.

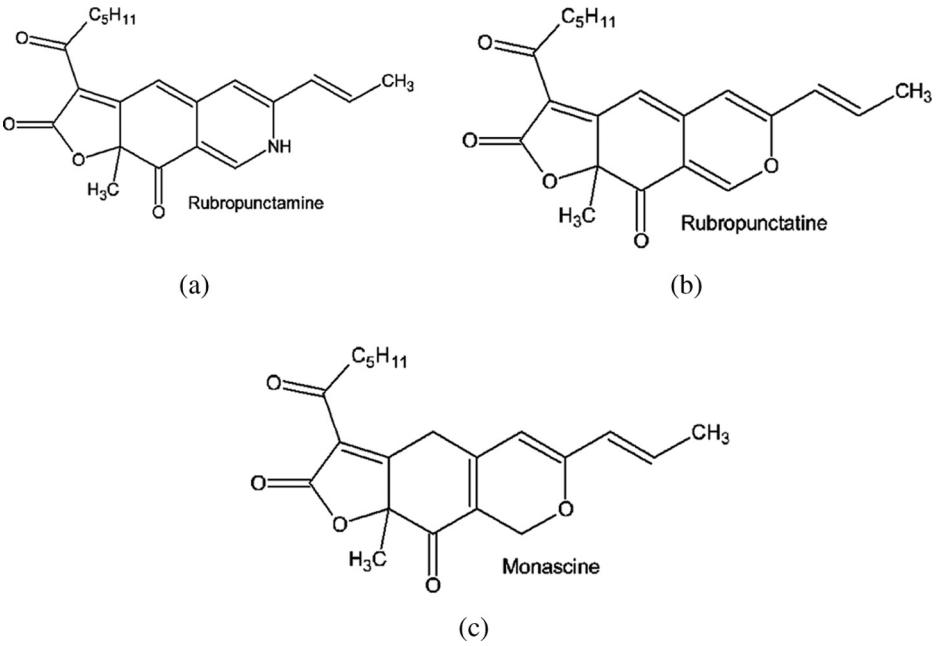

Figure 4. Chemical structure of the biopigments red (a), orange (b) and yellow (c).

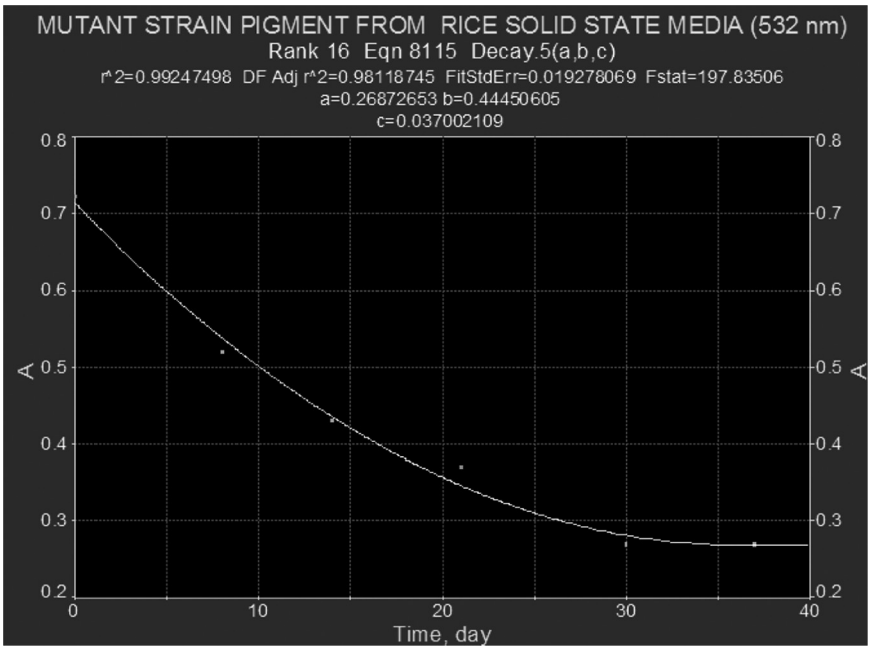

Figure 5. Stability of solid pigment obtained from rice, in solid-state media, measurement performed on the solid material at 532 nm. Fitting function realized by Table curve 2D.

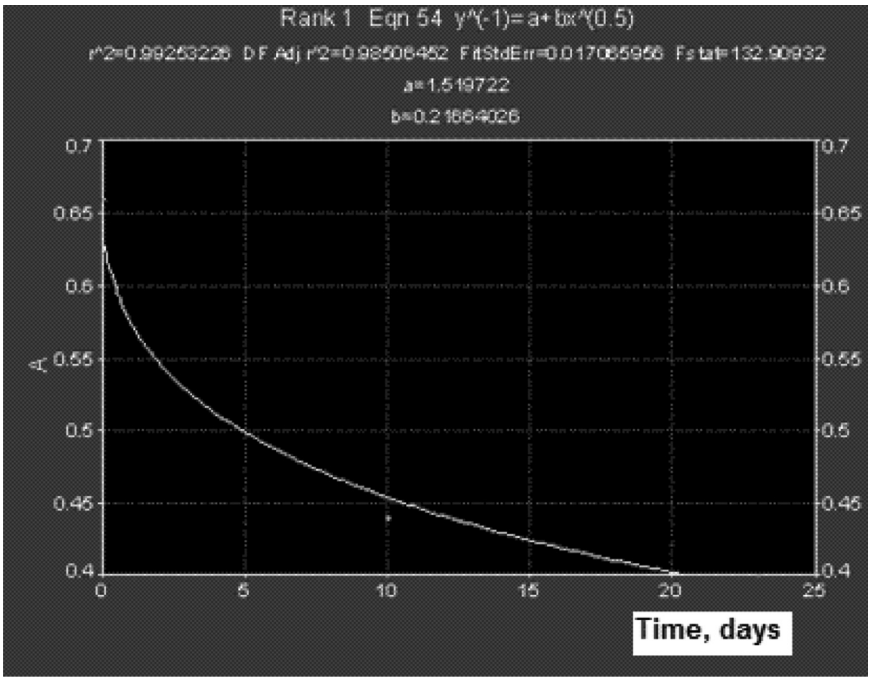

Figure 6. Stability of solid pigment obtained from rice, in methanol (red pigments). Fitting function edited by Table Curve 2D.

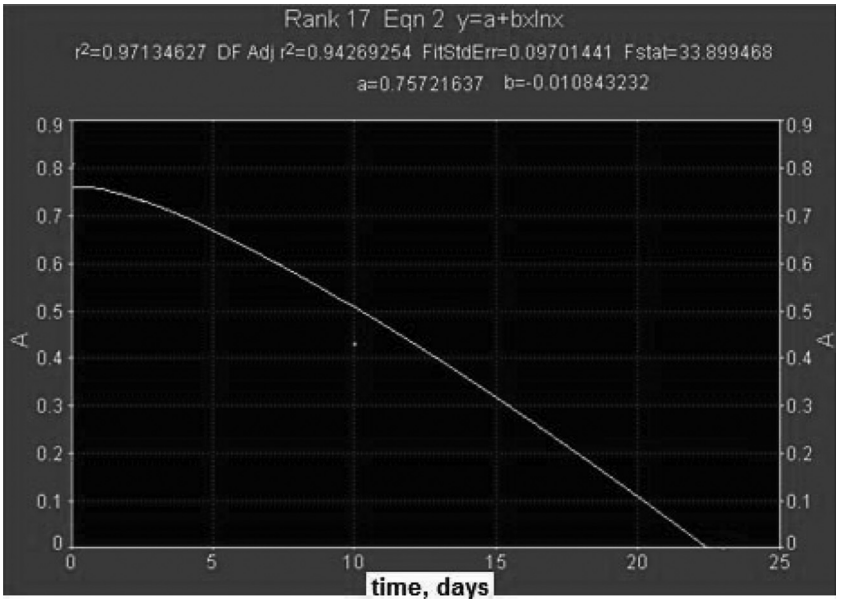

Figure 7. Stability of solid pigment obtained from rice, in acetone (orange pigments). Fitting function edited by Table Curve 2D.

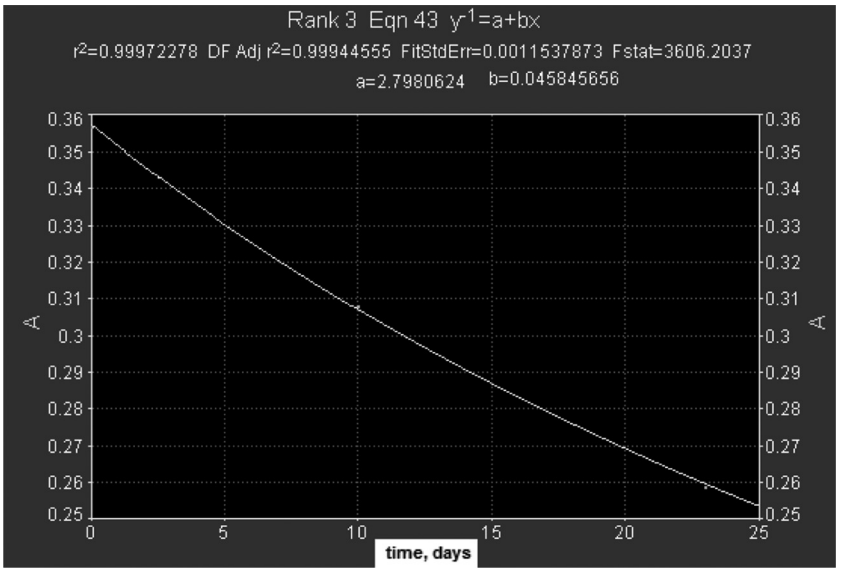

Figure 8. Stability of solid pigment obtained from rice, in N hexane (yellow pigments), measurement performed Fitting function done by Table Curve 2D.

Quantitative analysis of biopigment effectuated by atomic emission in inductively coupled plasma indicates a high content of C and N, H, P, K, Mg, Ca, Na and microelements (Table 2).

Thermal analysis indicates the stability of bioproduct in the range (25 – 74°C) (Fig. 12); after 75°C, biomaterial was decomposed, in the first stage by release

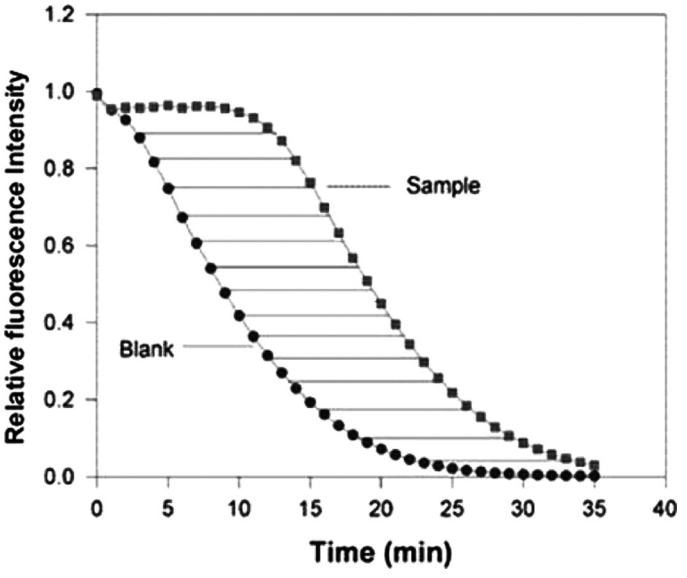

Figure 9. Antioxidant capacities of biomaterial, in fresh water.

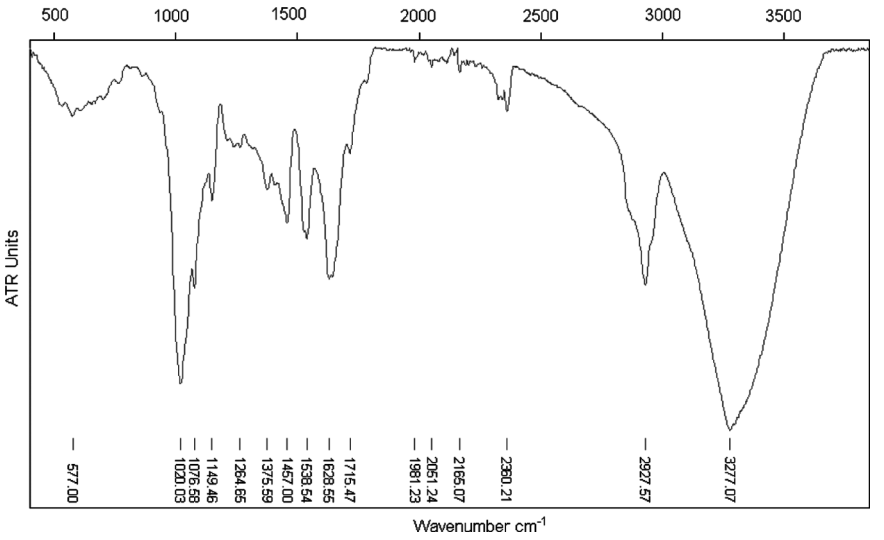

Figure 10. FT-IR spectrum of biomaterial performed in solid state by ATR.

inter or intramolecular water and then by organic material decomposition up to 700°C, when almost all biomaterials are transformed in NO_x, CO_x, and H_2O (99.425% loss weight).

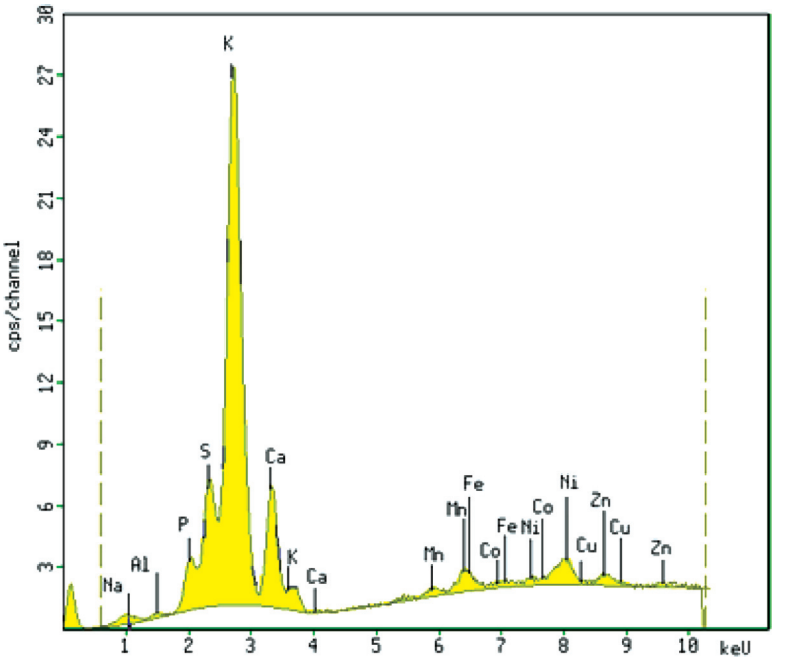

Figure 11. Fluorescent X-ray analysis of the solid biopigment.

Table 2. Elemental analysis of red pigment obtained in solid state

Element	%	Element	%
C	48.80	Mn	0.099
H	7.170	Fe	0.338
N	4.030	Mg	2.668
Ca	4.386	Na	1.520
P	14.936	Zn	0.708
K	13.572	S	0.036

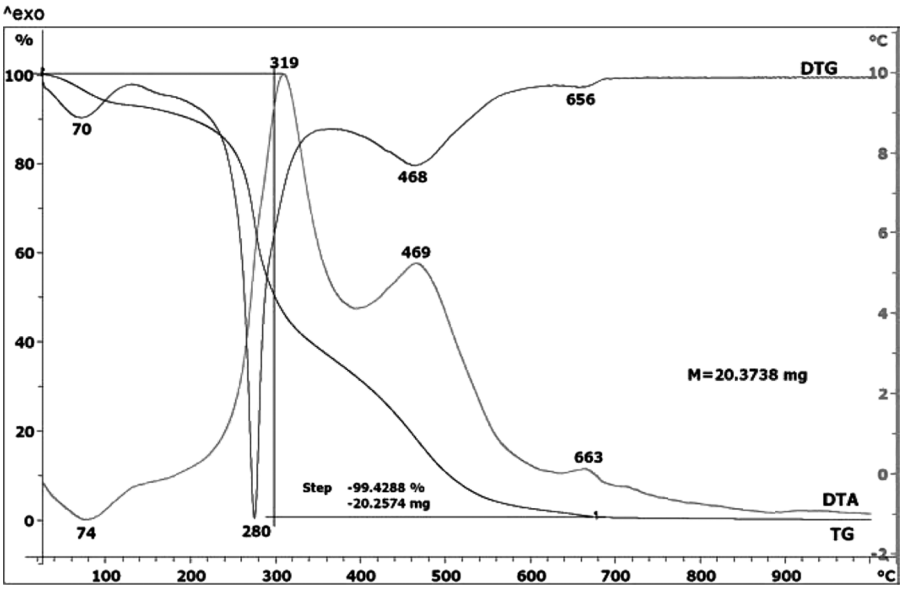

Figure 12. Thermal analysis of bioproduct obtained in solid state culture media.

Conclusions

Biosynthesis in the liquid and solid-state culture media reveal a great efficiency of the last media regarding the amount of pigment production. Solid pigment obtained in the solid state culture media, was strongly decomposed in 30 days while in the ethanol media (red, orange and yellow pigments extraction), the same biomaterial are decomposed in 20 days. In acetone (orange and yellow pigments extraction) and N hexane (yellow pigments extraction), the decomposition takes place in 23–25 days. In water, biopigments are strongly decomposed; but in the fresh solution the biopigment mixture reveal a powerful antioxidant capacity in comparison with E vitamin. The absorption of carbonyl, amide I and amide II presented in the infrared spectra of solid biomaterial demonstrates the presence of yellow, orange and red pigment in the biomaterial. Elemental analysis of these

materials indicates the presence of elements like C, H, N, Ca, P, K, Mg, Na, S, Fe Mn, Zn. Thermal analyses performed in order to test the thermostability of the bioproducts indicate a conditioning temperature below 70°C.

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