CONTRIBUTIONS TO EVALUATION OF THE BIODEGRADABILITY BY ASPERGILLUS NIGER AND OTHER FUNGI'S OF SOME INSULATING OILS

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Abstract - Mineral insulating oils used in electrical equipment because of their toxic organic substances and xenobiotic, represents a major risk to the environment - to accidental spills pollute soil, groundwater and surface water. By microbiological tests were evaluated the biodegradability of some insulating oils used in electrical equipment. The assays were performed in comparison with edible sunflower oil and with a control sample (culture medium - without oil). The experimental results indicate that the mineral oils are more readily biodegradable than synthetic ester oil and vegetable oils. It was also found that oils with high sulfur content are more readily biodegradable.

Keywords: insulating oils, mineral oils, synthetic esters, vegetal oils, biodegradability, molds.

1. INTRODUCTION

Insulating oils are widely used in electric equipment. Worldwide, only at the filling of transformers are used several billion liters of insulating oil [1]. Also, large amounts of insulating oil, billions of liters, are used to fill breakers, capacitors, power cables etc. Due to its physic - chemical properties (low dielectric permittivity and electrical conductivity, dielectric strength and high breakdown voltage, acceptable chemical stability etc.) and relatively low manufacturing costs, in these applications, are traditionally used mineral oils obtained by fractionated distillation of the oil.

Despite their advantages, the use of mineral oils in electric equipment, presents a number of limitations such as the relatively low flash point (approx. 130^0C), is obtained from non-renewable resources, limited compatibility with seals and/or other materials in contact during operation [2], soil and water in case of spills or accidental release [3]. In this context, in the perspective of sustainable development all over the world there is a constant concern, research aimed at replacing the insulating fluid applications of mineral oils with synthetic and / or natural ester oil [4 -9].

Microorganisms by their metabolism, and by the enzymes produced, initiates the degradation of non-metallic materials [10], thus producing both natural biodegradation of materials (process with positive effects for nature - provides global circulation of biogenic material in nature and diminishes the local storage of waste materials by their remineralization) and bio-deterioration of functional materials by the modification of the performance of the material, (conversion of a valuable material into waste). Biodeterioration is a process with negative effects for the economy, which should be avoided or delayed.

In other words, biodegradation is the biochemical process of decomposition of biodegradable materials into CO_2, methane, water, or biomass, a process in which the predominant mechanism is the enzymatic action of microorganisms, which can be measured by specific determinations in a defined period of time.

The biodegradation of the insulating oils is due to the synergistic action [11] of bacteria [12] and fungi [13].

To determine the biodegradability of oil products in wet environments were developed several international standardized methods (by ex. CEC -L- 103-12 [14], CEC L -33- T -82 - subsequently reformulated as new CEC L - 33 -A -93 , etc.) and national standards (EN ISO 7827 : 2013 [1], SR EN ISO 9408 : 2004 [16]), methods that are based on the determination of the amount of the carbon in the pollutant which is metabolized during the determination - as is required by regulation (usually more than 21 days). Application of these rules presents some disadvantages (complex equipment, relatively long duration of measurements etc.) [17, 18], issues that have initiated the development of new regulations [19].

The biodegradability is directly related with the resistance by the action of microorganism's materials. The resistance to the action of microorganisms is determined by specific microbiological tests which aims the ability to form biofilms of microorganisms on the surface of the investigated material [20, 21], methods whose application is simple, do not require special features and which allows a relatively rapid evaluation. Another advantage of these methods lies in the fact that, depending on the inoculum used, allows the determination of the resistance to the action of a single species and/or simultaneous and synergistic action of several species (mixed cultures).

Aspergillus niger is a filamentous fungus ubiquitous

in nature, presents a wide geographical distribution, which is due to the great tolerance to extreme environmental conditions. It is xenotolerant, it develops in a wide range of temperatures (10 - 50^0C) and pH (2-11), even in extreme salinity environments (up to 34 %). Is highly resistant to herbicides products, pesticides - including toxic salts of heavy metals, which they adsorb from the environment. Asexual spores can withstand extreme environmental conditions (freezing, excessive heat, pH variations) and allows the body to survive in the inactive periods [22].

In this context, *the aim of this paper* is to assess the biodegradability of insulating fluid (mineral transformer oils and ester) in both media inoculated with spores of *Aspergillus niger* environments inoculated simultaneously with several species of mold.

2. EXPERIMENTAL PART

The biodegradability of insulating oils was evaluated by comparative measurements which aimed mold growth on oil stains applied to the culture media type Czapek-Dox (mineral salt solution buffered, gelled by adding agar) inoculated with spores of molds. The culture medium was prepared from Merck reagent quality by dissolving in 1000 ml distilled water to 2g $NaNO_3$,; 0.7g KH_2PO_4; 0.3g K_2HPO_4; 0.5g KCl; 0.5g $MgSO_4 \cdot 7\ H_2O$; 0.01g $FeSO_4$ and 10g agar-agar.

The measurements were performed using two methods: Method A (Czapek- Dox incomplete medium without carbon source) and method B (Czapek - Dox complete medium - carbon source 30g sucrose / 1000ml). The biodegradability was evaluated by determining the density of fructifications or counting data fructifications surface (fructifications / mm^2 culture medium covered with oil).

On the sterile culture medium was added 300µl sample of oil, then it was inoculated by spraying a spore of homogeneous suspension (approx. 10^5 spores / ml). The measurements were made using both pure inoculum of *Aspergillus niger* spores and with inoculum mixed spore with *Myrothecium verrucaria, Paecilomyces variotii, Trichoderma viridae, Chaetomium globosum, Aspergillus ustus, Penicillium citrinum, Aureobasidium pullulans, Alternaria alternata, Cladosporium herbarum, Aspergillus flavus, Scopulariopsis brevicaulis* and *Aspergillus niger.*

Were evaluated the biodegradability's of the new transformer oil samples having mineral origin, unused (Nytro Taurus product produced by Nynas and TO 30.01 produced by MOL) and depleted (recovered during overhaul of power transformers of 400kV Filiaşi - operating for 34 years, Craiova 1MVA - operating for 27 years and Roman 630kVA - operating for 30 years) filled in the commissioning time with oil produced in RO) and ester oil samples - both synthetic type 205 Tri Luminol L Drum (manufacturing PETRO - Canada) and natural oil type BIOTEMP (produced by ABB - USA). The measurements of the biodegradability of electrical household oil samples were made in comparison using edible sunflower oil (refined) with a blank test (oil-free culture medium).

Because the oil biodegradation may be influenced by their content of sulphur (usually heterocyclic substances with sulfur that presents xenobiotic character [23-25]), for the investigated oils it was determined the content of sulfur using of X-ray fluorescence spectrometry (XRF) technique with an equipment type S8 Tiger (Bruker Germany).

Control samples (without oil) and the oil samples were incubated at 30 ± 2°C temperature with relative humidity of the air 90 ± 5% in the dark. Samples were periodically analyzed at 24, 48, 72 and 168 hours both macroscopic and microscopic (stereomicroscope). To assess the biodegradability were determined the degrees of coverage of the oil spill by counting fructifications mold colonies per unit area (fructifications/mm^2). For each determination were counted the fructifications on 5 squares of 1mm^2, and the values obtained were averaged.

3. EXPERIMENTAL RESULTS AND THEIR INTERPRETATION

The results concerning the sulphur content in the oil investigated samples are shown in Table 1.

Table 1. The sulfur content of the oil samples

Type of oil	Manufacturer	Sample	S [%]
mineral	România 1980	Filiaşi	0,14
	România 1983	Roman	0,15
	România 1986	Craiova	0,14
	MOL Hungary	TO 30.01	0,04
	NYNAS	*Nytro Taurus*	0,04
syntethic	PETRO - Canada	*Luminol Tri 205 L Drum*	≤0,0001
vegetal	ABB USA	Biotemp	≤0,0001
	EXPUR S.A. „Bunica" RO	Sunflower	≤0,0001

Analyzing the values shown in Table 1. resulted that the used oil samples have a relatively high sulphur content (approx. 0.15 %) in comparison with 0.04 % as it had at the date of commissioning, which may be due to the contact during operation time with vulcanized rubber seals. It results also that the oil based on synthetic ester (Luminol Tri 205 L Drum) and the vegetable oils investigated presents a sulpfur content below the detection limit of the equipment S8 Tiger (0.0001 %).

The results of the microbiological observations are summarized in Table 2. Table 3 shows the average density of fructifications (as a measure of the coverage with mold and default biodegradability) on oil samples investigated.

Figures 1-6 shows representative images to illustrate the observations from Table 2 .

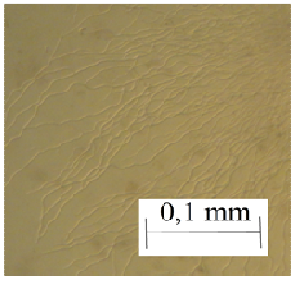

Fig 1. Oil sample Mol- 48 hours, *Aspergillus* niger, complete media (Hyphae well developed)

Table 2. The results of microbiological observations

Oil sample	Incubation time [hours]	Pure culture *Aspergillus niger*		Mixed culture	
		Average density of fructifications [no/mm^2]			
		Media "A" without sucrose	Media "B" with sucrose	Media "A" without sucrose	Media "B" with sucrose
FILIAȘI (34 years in use)	24	Does not present growth	Does not present growth	Does not present growth	Does not present growth
	48	Does not present growth	Hyphae poorly developed	Does not present growth	Poor growth (hyphae)
	72	Hyphae poorly developed, small	Small conidiophores with poorly developed fructifications	Hyphae poorly developed	Small conidiophores with poorly developed fructifications
	168	Small conidiophores with poorly developed fructifications	Conidiophores with young fructifications, rarely mature	Small conidiophores with poorly developed fructifications	Conidiophores with young fructifications, rarely mature; predominant species: *Aspergillus niger,Trichoderma viridae, Paecilomyces variotii, Penicillium sp.*
ROMAN (30 years in use)	24	Does not present growth	Does not present growth	Does not present growth	Does not present growth
	48	Does not present growth	Poor growth (hyphae)	Does not present growth	Small hyphae, poorly branched
	72	Poor growth (hyphae)	Small conidiophores with poorly developed fructifications	Poor growth (hyphae)	Small conidiophores with poorly developed fructifications
	168	Small conidiophores with poorly developed fructifications	Young fructifications, rarely mature	Small conidiophores with poorly developed fructifications;	Relatively rare mature fructifications; predominant species: *Aspergillus niger,Trichoderma viridae, Penicillium sp.*
CRAIOVA (27 years in use)	24	Does not present growth	Does not present growth	Does not present growth	Does not present growth
	48	Does not present growth	Hyphae poorly developed	Does not present growth	Hyphae poorly developed
	72	Poor growth (hyphae)	Small conidiophores with poorly developed fructifications	Poor growth (hyphae)	Small conidiophores with poorly developed fructifications
	168	Small conidiophores with poorly developed fructifications, rarely mature	Rarely mature fructifications, poorly developed	Small conidiophores with poorly developed fructifications, rarely mature	Young fructifications, rarely mature; predominant species: *Aspergillus niger , Trichoderma viridae, Penicillium sp.*
MOL TO 30.01	24	Does not present growth	Poor growth (hyphae)	Does not present growth	Hyphae poorly developed
	48	Hyphae poorly developed	Hyphae well developed	Hyphae poorly developed	Hyphae well developed
	72	Small conidiophores with poorly developed fructifications	Large conidiophores, young and mature fructifications	Small conidiophores with poorly developed fructifications	Conidiophores with mature fructifications, rarely young
	168	Rarely mature fructifictions	Rarely mature fructifications	Young fructifications, poorly developed, rarely mature	Rarely mature fructifications - species: *Aspergillus niger,Trichoderma viridae, Paecilomyces variotii, Penicillium sp.*
NYNAS *Nytro Taurus*	24	Does not present growth	Does not present growth	Does not present growth	Does not present growth
	48	Hyphae poorly developed	Hyphae well developed	Hyphae poorly developed	Hyphae well developed, branched
	72	Small conidiophores with poorly developed fructifications	Large conidiophores, good developed, young fructifications	Small conidiophores with poorly developed fructifications	Large conidiophores, good developed, young fructifications
	168	Large conidiophores, poorly developed fructifications,	Relatively rare mature fructifications	Rarely fructifications, poorly developed	Rarely mature fructifications
LUMINOL Tri 205 L Drum	24	Poor growth (hyphae)	Hyphae poorly developed	Poor growth (hyphae)	Small hyphae, branched
	48	Small conidiophores with poorly developed fructifications	Small conidiophores with poorly developed fructifications	Small conidiophores with poorly developed fructifications	Conidiophores with young fructifications; predominant species: *Aspergillus niger , Trichoderma viridae, Penicillium sp.*
	72	Conidiophores with young fructifications	Mature fructifications, rarely young	Small conidiophores with poorly developed fructifications	Large conidiophores, young fructifications, rarely mature
	168	Young fructifications, poorly developed, rarely mature	Mature fructifications	Young fructifications, poorly developed, rarely mature-species: *Aspergillus niger, Paecilomyces variotii, Penicillium sp.*	Mature fructifications; predominant species: *Aspergillus niger, Trichoderma viridae, Paecilomyces variotii, Penicillium sp.*
BIOTEMP	24	Does not present growth	Does not present growth	Does not present growth	Does not present growth
	48	Poor growth (hyphae)	Poor growth (hyphae)	Poor growth (hyphae)	Poor growth (hyphae)
	72	Small conidiophores with poorly developed fructifications	Hyphae well developed, small conidiophores, poorly developed fructifications	Small conidiophores with poorly developed fructifications	Small conidiophores with poorly developed fructifications
	168	Rarely fructifications, poorly developed	Young fructifications, relatively rare	Conidiophores with young fructifications	Large conidiophores, young fructifications, rarely mature
SUN FLOWER (refined)	24	Hyphae well developed	Hyphae well developed, branched	Hyphae well developed	Hyphae well developed, branched
	48	Large conidiophores, young fructifications	Large conidiophores, young fructifications	Large conidiophores, young fructifications	Large conidiophores, young fructifications
	72	Young fructifications, rarely mature	Mature fructifications, rarely young	Young fructifications, rarely mature	Mature fructifications, rarely young; predominant species: *Aspergillus niger, Paecilomyces variotii, Penicillium sp.*
	168	Mature fructifications, well developed	Mature fructifications, well developed	Mature fructifications - predominant species: *Aspergillus niger, Trichoderma viridae, Paecilomyces variotii, Penicillium sp.*	Mature fructifications, well developed; predominant species: *Aspergillus niger, Trichoderma viridae, Paecilomyces variotii, Penicillium sp.*
CONTROL SAMPLE (without oil)	24	Small hyphae, branched	Small hyphae, branched	Small hyphae, branched	Small hyphae, branched
	48	Small conidiophores with young fructifications	Small conidiophores young fructifications	Small conidiophores with young fructifications	Small conidiophores with young fructifications
	72	Mature fructifications, rarely young	Mature fructifications, rarely young	Mature amd young fructifications	Mature fructifications, rarely young
	168	Mature fructifications	Mature fructifications	Mature fructifications – predominant: *Aspergillus niger, Trichoderma viridae, Paecilomyces variotii, Penicillium sp.*	Mature fructifications - predominant species: *Aspergillus niger, Trichoderma viridae, Paecilomyces variotii, Penicillium sp*

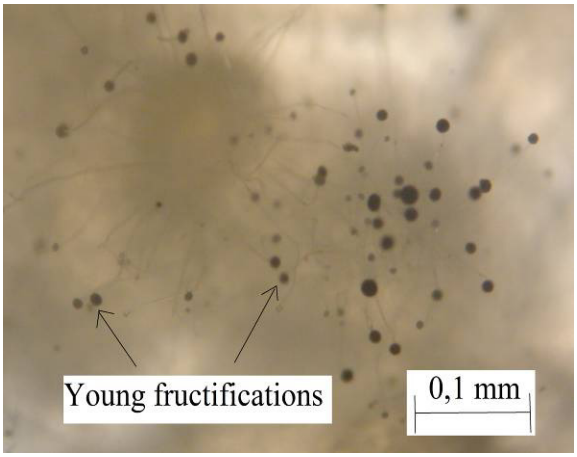

Fig. 2. Oil sample Filiaşi, mixed culture, incomplete media, *Aspergillus niger* (Conidiophores with young fructifications, poorly developed)

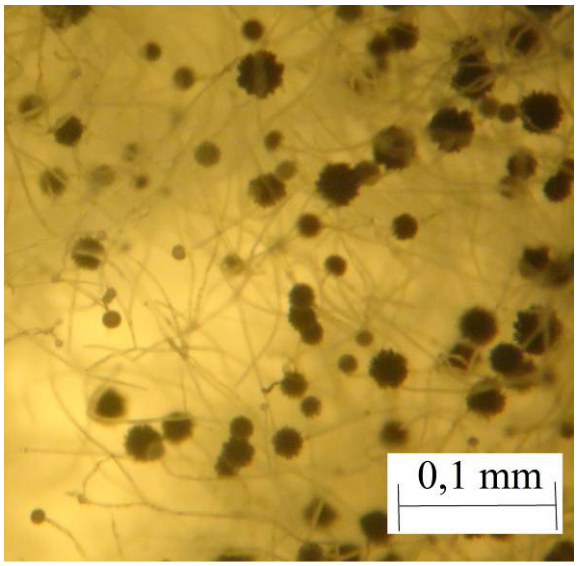

Fig. 3. Oil sample Nynas-72 hours, *Aspergillus niger*, complete media (Conidiophores with young and mature fructifications of *Aspergillus niger*)

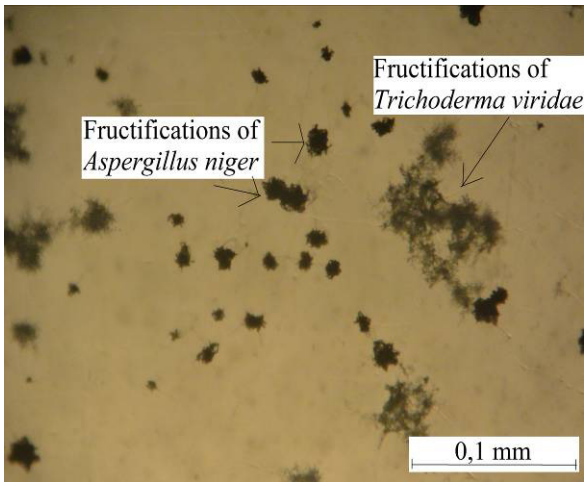

Fig. 4. Oil sample Luminol-48 hours, mixed culture, complete media (Fructifications of *Aspergillus niger* and *Trichoderma viridae*)

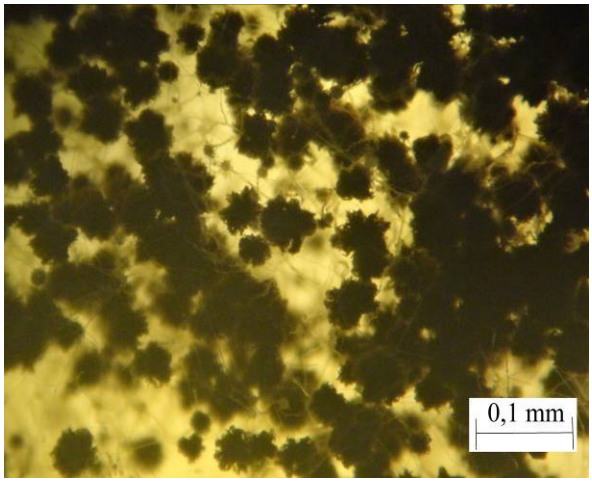

Fig. 5. Oil sample sun flower-168 hours, *Aspergillus niger*, incomplete media (mature and dense fructifications of *Aspergillus niger*)

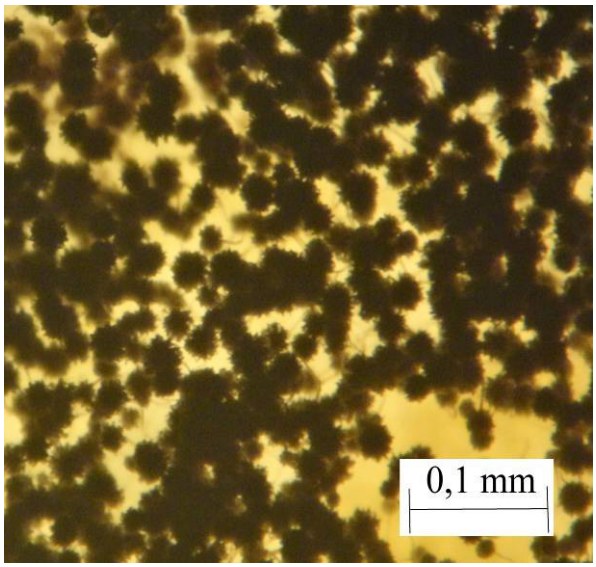

Fig. 6. Control sample, 72 hours, incomplete media, *Aspergillus niger* (Mature fructifications, very well developed)

Analyzing the data presented in Table 2 and Table 3 it is noted that the content of sulphur of the oil samples highly determines their biodegradability. So, the used oil samples, taken from Filiaşi, Craiova and Roman transformers after approx. 30 years of operation, having the sulphur content up to 0.14 to 0.15 %, are most readily biodegradable. On these samples the first signs of growth appear only after 72 hours of incubation - and only then on average slightly assimilability carbon source (sucrose). Similar behavior has been found - against expectations [26] – also on Biotemp oil, fact that can be explained by the content of the antioxidants additives and corrosion inhibitors that those oils contain. These findings suggest that these samples contain toxic substances having a xenobiotic effect, which extends the LAG period in which the synthesis takes place suitable for the metabolization of the enzyme's source of food.

Table 3. The evolution of the average density of fructifications mold in the investigated oil samples

Sample of oil	Exposing time [hours]	Pure culture *Aspergillus niger*		Mix culture	
		Average density of fructifications [nr/mm^2]			
		Medium "A" without sucrose	Medium "B" with sucrose	Medium "A" without sucrose	Medium "B" with sucrose
FILIAŞI (blown)	48	0	0	0	0
	72	0	3	0	4
	168	4	14	5	16
ROMAN (blown)	48	0	0	0	0
	72	0	2	0	5
	168	3	11	4	13
CRAIOVA (blown)	48	0	0	0	0
	72	0	4	0	6
	168	5	15	6	17
MOL TO 30.01	48	0	1	0	1
	72	2	18	4	25
	168	11	26	13	31
NYNAS Nytro Taurus	48	0	0	0	0
	72	1	4	2	9
	168	6	18	7	22
LUMINOL Tri 205 L Drum	48	1	5	2	8
	72	4	19	6	22
	168	10	35	14	42
BIOTEMP	48	0	0	0	0
	72	2	14	4	15
	168	8	27	10	31
FLOAREA-SOARELUI (refined)	48	8	16	9	19
	72	19	42	20	44
	168	98	156	101	160
MARTOR (medium without oil)	48	12	23	16	26
	72	25	57	27	61
	168	146	178	123	185

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In the case of sunflower oil (vegetable triglyceride) and Luminol Tri 205 L Drum oil (synthetic ester) are detected that on the exposed samples on both sucrose - free medium and complete sucrose medium, at only 48 hours after inoculation appear the first fructifications.

Comparing the data recorded on samples inoculated with spores of *Aspergillus niger* only (pure culture) with the ones with inoculum mixed (mixed culture), there was not found significant differences, suggesting that the contribution of the *Aspergillus niger* mold to the biodegradation of the investigated oil samples is decisive.

Considering the average density of the fructifications grown on oil samples as a measure of the biodegradability, from the data of the Table 3 it is set out a hierarchy of the biodegradability of oil samples investigated as follows: Sunflower > Luminol Tri 205 L Drum > Biotemp≈ MOL TO 30.01 > Nytro Taurus> Craiova ≈ Filiaşi > Roman - as illustrated in Figure 7.

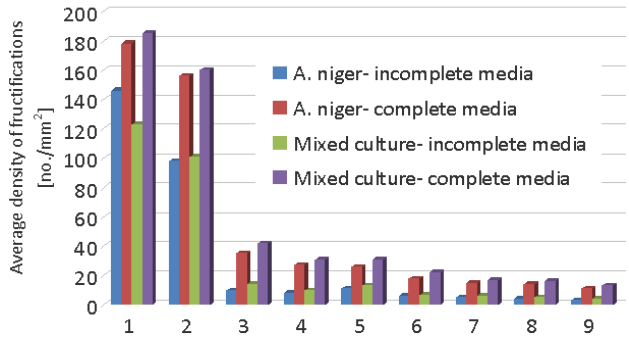

Fig. 7. Biodegradability using mixed molds culture of investigated oil samples (exposure time 168 hours): 1- sample (without oil); 2 – sunflower; 3 – Luminol Tri 205 L Drum; 4 – Biotemp; 5 – MOL TO 30.01; 6 – Nytro Taurus; 7 – Craiova; 8 – Filiaşi; 9 – Roman

4. CONCLUSIONS

Using specific microbiological tests it was evaluated the biodegradability by the action of molds of different sorts of transformer oil, in comparison with a sample of refined sunflower oil and an oil-free blank in the culture. After processing the experimental dates, the main conclusions are:

- For mineral oils, increasing the Sulphur content leads to a lower biodegradability, which can be explained by the action of xenobiotic Sulphur compounds contained in the composition of these oils;
- The content of antioxidants additives and corrosion inhibitors existing in Biotemp oil decreases substantially the biodegradability due to the molds (to sunflower oil - without additives);
- The biodegradability of ester based synthetic oil is similar to the one of the mineral oil of low Sulphur content;
- The biodegradability by the action of molds of refined sunflower oil (natural triglyceride) is approx. 4 times greater than the one of the synthetic ester oil and approx. 9 times greater than mineral oils.

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