Polybutylene succinate preparation and Biodegradation study of cellulose and cellulose blends

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***Abstract*— The humidity and the quantity of water are well-known factors affecting the results of biodegradability tests. Experiments are conducted with blend of biopolymers (cellulose/polybutylene succinate), blend of natural polymer and synthetic polymer (cellulose/polycaprolactone) and cellulose only. The American Testing material (ASTM) Standard was the method chosen to evaluate the ultimate aerobic biodegradability. Inoculum was obtained from the lixivia extracted from the public discharge of Oujda city (Morocco).The percentage of microorganisms growth in surface of samples was followed in differentes environnements: contamination, inoculation at low humidity and inoculation at height humidity. From the tests of contamination and inoculation at low humidity environnements, microorganisms growth in surface of samples was favored in case of contamination, followed but not so much by the inoculation. We noted A degrease of microorganisms growth in surface of samples in case of inoculation at height humidity environment .We concluded that humidity retard the rate of biodegradation (cellulose, cellulose /biodegradable polymers).**

***Index Terms*— biodegradability, cellulose, polybutylene succinate, polycaprolactone, blends.**

I. INTRODUCTION

Each year more than 100 billion tons of organic matters are produced by photosynthetic organism (plant, algae, and some bacteria) [1] that the half of mass form is cellulose [2]. This polymer, normally branches with hemicellulose and lignin, has to undergo unhealthy chemical process with harsh alkali and acid treatment to improve its properties for industry. Thus to preserve the health and the environment we usually used one of the most efficient and economically profitable methods, which is the creation of green composite materials, via mixing of synthetic or natural and natural polymers. There is limited number of works in literature dedicated to investigation of cellulose blends with various synthetic polymers, for example nylon-6 is incompatible with cellulose, and poly (ε-caprolactam) is partially compatible [3]. Cellulose-polyamide blends are heterogeneous two-phase systems [4], whereas a blend of cellulose with poly (4-vinylpyrrolidone) is homogeneous at any ratio [5]. The investigation of the films obtained from blends of cellulose with poly (ethylene terephthalate) showed that a specific polymer-polymer interaction takes place [5].

Recently, polycaprolactone (PCL) and polybutylene succinate (PBS) attract more attention because PCL combines biodegradability and compatibility [6-11] and PBS is a biodegradable polymer, its monomers [succinic acid, (1-4) butandiol] can be obtained from biomass by fermentation, [12-20].

Green composites obtained by blending cellulose and PCL [21-23] or PBS [24] were much investigated especially for their good thermal and mechanical proprieties. The biodegradation rates of the green composites need to be evaluated when deciding suitable waste management methods and for designing future waste disposal facilities. However there is a little information on the biodegradation characteristic of this green composite.

The main goal of this work is:

- Synthesis of green composites widely used in industry having differentes compositions.
- Evaluation of their biodegradation in some environments.

II. EXPERIMENTAL

Cellulose (Mn = 300 g/mol), polycaprolactone (Mn = 10000 g/mol), (1,4) butanediol, succinic acid, paratoluene sulfonic acid, titanium butoxide, monopotassium phosphate, potassium hydrogen phosphate, magnesium sulfate heptahydrate, ammonium nitrate, sodium chloride, ferrous sulfate heptahydrate, zinc sulfate heptahydrate, manganese Sulfate heptahydrate, dichloromethane, toluene and ether were purchased from Sigma–Aldrich Chemical Co. All reagents were used as received. Lixivia is recuperated from landfill site of Oujda city (Morocco).

2.1. Fourier transforms infrared spectroscopy (FTIR)

The FTIR spectra were recorded using IRTF B8400S Shimadzu between 4000 and 600 cm^{-1} at resolution of 4 cm^{-1}, potassium bromide is used as the background.

2.2. Nuclear magnetic resonance (^{1}H-NMR)

The ^{1}H-NMR spectra were recorded in UATARS - CRNST – RABAT- MOROCCO using spectrometer BRUKER. The deuterated chloroform ($CDCL_3$) with trifluoroacétic acid ($C_2HF_3O_2$) were used as solvent and tetramethylsilane (TMS) as internal reference.

2.3. Differential scanning Calorimetric (DSC)

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Test differential scanning calorimetry analysis (DSC) is performed by a TA DSC Q20 (United State). About 10 mg of sample was placed in sealed aluminum capsules. Samples were subjected to two scan from - 40 to 200 °C with a rate of 10 °C /min. The crystallinity degree (Xc), was Calculated from:

$$Xc\ (\%) = \frac{\Delta H_m}{(w_{poly} \times \Delta H_{100}) \times 100}$$

- ΔH_m: Polymer melting enthalpy calculated from second scan,
- W_{poly}: Weight fraction of Polymer in blend (20%)
- ΔH_{100}: Melting enthalpy of 100% cristallin Polymer

2.4. Synthesis of polybutylene succinate

The polybutylene succinate was synthesized in two stages the first by melt condensation using (1, 4) butanediol, succinic acid and paratoluene sulfonic acid (APTS). (1, 4) butanediol (0.01 mol), succinic acid (0.19 mol) and APTS (0.1%) were introduced in three-necked round bottom flask and 20 ml of toluene was added. A thermometer was fitted to the neck, a stirrer to the other and Dean Stark was fitted to third neck. The temperature was kept at 115 °C for 24 hours to remove water by azeotropie. The organic solvent was extracted and 0.1% of Titanium Butoxide was added as a catalyst with constant stirring, the temperature was carried out at 240 °C under vacuum for another 6 hours. The reaction mixture was dissolved in dichloromethane and precipitate in an excess of ether, the white product was removed by filtration and kept at 60 °C under vacuum for 24 hours.

2.5. Preparation of the cellulose blends.

An amount of cellulose (80%) and Polybutylene succinate (20%) was added to dichloromethane under constant stirrer during 3 hours at room temperature and the mixture was removed in Petri dish, the solvent was eliminated by evaporation over the night and then kept in desiccators with P_2O_5 powder for 24 hours under vacuum.

2.6. The aerobic biodegradability test methods

For tests in the solid phase, we used the standard procedures ASTM G 21-90 and ASTM G 22-76, these standards specify a method by determining the resistance of polymeric materials to bacteria and fungi [25]. The aim of these tests is to see if micro-organisms can colonize the polymer surface and use it as a sole carbon source for their growth.

These tests were performed in Petri dishes containing a mineral medium (M_1) which is composed of monopotassium phosphate (KH_2PO_4: 0.7 g); potassium hydrogen phosphate (K_2HPO_4: 0.7 g); magnesium sulfate heptahydrate ($MgSO_4/7H_2O$: 0.7 g); ammonium nitrate (NH_4NO_3: 1 g); sodium chloride (NaCl: 0.005 g); ferrous sulfate heptahydrate ($FeSO_4/7H_2O$: 0.002 g); zinc sulfate heptahydrate ($ZnSO_4/7H_2O$: 0.002 g); manganese Sulfate heptahydrate ($MnSO_4/7H_2O$: 0.001 g) dissolved in sufficient distilled water to make up 1000 ml. This medium was sterilized by autoclaving at 121°C for 20 min. After sterilization, the pH was between 6.0 and 6.5.

We placed the material tested on the agar medium M_1 and then we inoculated it with the lixivia extracted from the public discharge of Oujda city (Morocco) in petri dishes. The inoculum prepared was 1/3 of lixiviat diluted in 2/3 of sterilized physiologic water to reduce carbone in lixivia; the presence of homogenous microorganisms (bacteria, fungi…) was confirmed by microscopic observation. A medium M_1 inoculated with lixiviat without source of carbone was used as control.

2.6.1. The aerobic biodegradability by natural contamination.

Cellulose, blend of cellulose (80%) with polybutylene succinate (20%) and blend of cellulose (80%) with polycaprolactone (20%) were placed on the agar medium M_1. The petri dishes opened and left for 7 days in place near discharge of Oujda city (Morocco). The petri dishes were closed again and then incubated at 30 °C ± 2°C, for 28 days.

2.6.2. The aerobic biodegradability by inoculation

Cellulose, blend of cellulose (80%) polybutylene succinate (20%) and blend of cellulose (80%) polycaprolactone (20%) were placed on the agar medium M_1.We inoculated the surface by two drops of lixivia, the first drop in the middle of the sample, the second drop between the sample and the mineral medium M_1. The petri dishes were closed and then incubated at 30 °C ± 2°C, for 28 days.

2.6.2.1. The aerobic biodegradability at low humidity by inoculation

The inoculated samples were incubated at 30 °C ± 2°C and relative humidity between 10% and 20%.

2.6.2.2. The aerobic biodegradability at height humidity by inoculation

The inoculated samples were incubated at 30 °C ± 2°C at 75 % relative humidity.

2.6.3. Standard procedures ASTM G 21-90 and ASTM G 22-76 applications

The surface of polymers was observed visually and with microscope to evaluate their colonization by microorganisms. The rate of colonization of the polymer was evaluated as surface of polymer covered by colonies of microorganisms (**Table1)** :

Table.1. Observed Growth on Specimens and rating.

Growth	Rate
No growth	**0**
Traces of growth (less than10%)	**1**
Slight growth (10% to 30%)	**2**
Medium growth (30% to 60%)	**3**
Heavy growth (60% to complete coverage)	**4**

The surface of polymers was observed during 28 days to establish the growth percentage on the surface of samples, and if there was a visual change or not. Observation for visible effects and rate as follows (**Table1)**.Therefore the growth graph obtained as taking time (days) for X axis and growth percentage on the surface of samples for Y axis.

III. RESULTS AND DISCUSSION

3.1. Characterization of polybutylene succinate synthesized

3.1.1. FTIR analysis

From FTIR spectrum of polybutylene succinate presented in **(Figure.1)**, the absorption band appeared at 2947.82 cm^{-1} is assigned to the C–H stretching bonds of the polymer. The band at 1716.78 cm^{-1} is assigned to C=O stretching vibration of the ester carbonyl group, the band at 1341.25 cm^{-1} is assigned to –COO- bond stretching vibration. The signal at 1158.73 cm^{-1} is characterizing the C–O–C stretching vibration in the repeated $–OCH_2CH_2$ units.

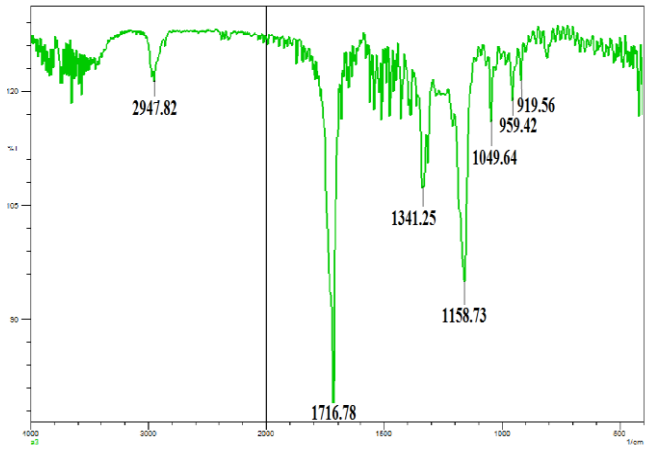


Figure.1. FTIR spectrum of polybutylene succinate

3.1.2. ^{1}H-NMR analysis

^{1}H-NMR spectrum of polybutylene succinate is shown in (**Figure.2).** The peak at 2.65 ppm is attributed to methylene protons on succinic acid unit (a). The peak at 4.3 ppm is attributed to methylene protons O=C-O-$\underline{CH_2}$-CH_2-CH_2-$\underline{CH_2}$-O-C=O (c) on butanediol unit, and the peak at 1.6 ppm is attributed to the centered methylene protons (b) of (1,4) butanediol. The protons of the methylene in α position of the hydroxyl group at the chain end (d) appeared at 3.35 ppm. This signal is used to determine the molecular weight of the PBS prepared.

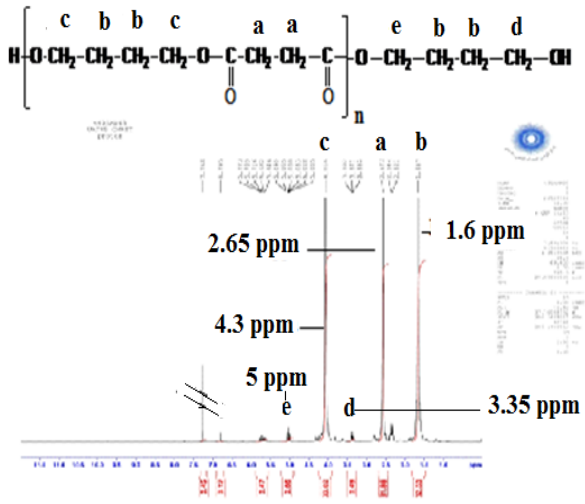


Figure.2. ^{1}H-NMR of the polybutylene succinate

3.1.3. Differential scanning calorimetric of PBS

As showed in **(Figure.3),** the main melting peaks are at T_{m2} = 112 °C in case of the first run and T_{m1} = 111 °C for the second run. The main crystallization peaks for the first and the second run are relatively obtained at temperature range around T_{c2} = 62.5 °C. In second scan we observed a little crystallization peak at T_{c1} = 90.2 °C, followed by Pre-melting temperature at T_{pm} = 100 °C. The glass transition temperatures was observed at the beginning of the experiment at T_g = - 40 °C.

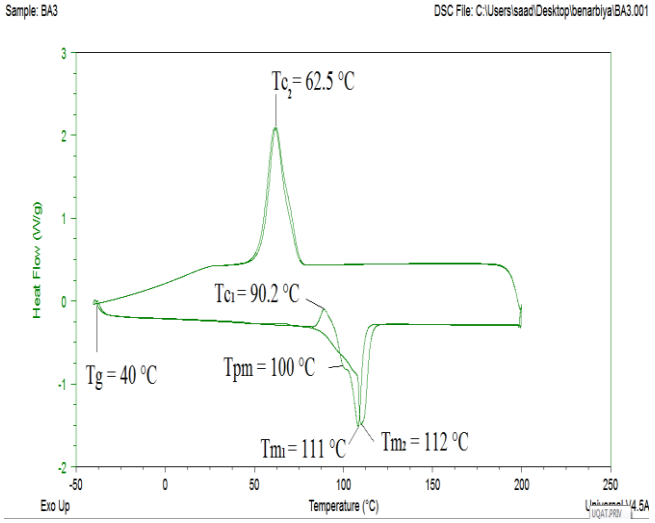


Figure.3. Differential scanning calorimetric of PBS

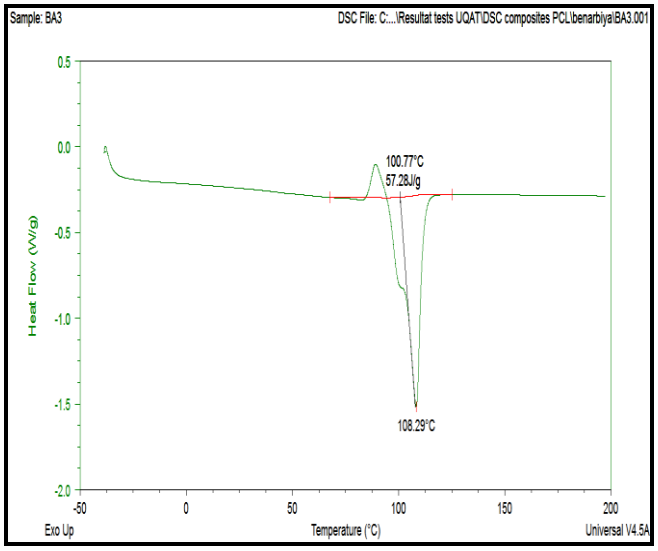


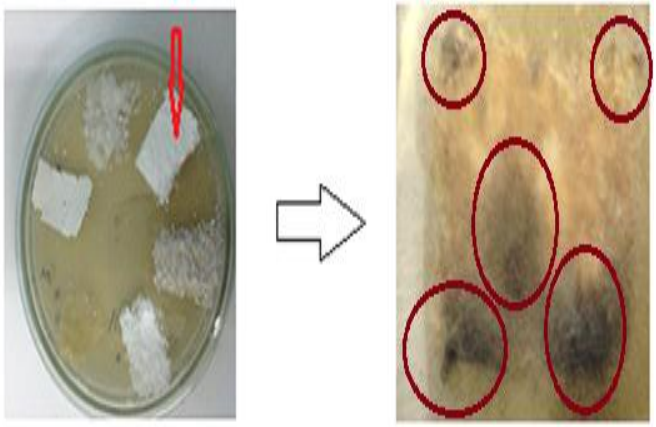
Figure.4. The melting enthalpies of the polybutylene succinate determined in the second scan

Table.2. proprieties of polybutylene succinate prepared.

T_g (°C)	T_{m2} (°C)	ΔH_f J/g	X_c (%)	M_n (g/mol)
- 40 °C	112	57.28	25.85	3010

3.2. Biodegradation of cellulose

3.2.1. Cellulose biodegradation by natural contamination



Cellulose initiated state

Cellulose after 28days, (heavy growth)

Figure.5. Cellulose biodegradation by contamination.

3.2.2. Cellulose biodegradation by inoculation with lixivia at low humidity

Cellulose initiated state

Cellulose after 28 days, (medium growth)

Figure.6. Cellulose biodegradation by inoculation with lixivia low humidity.

3.2.3. Cellulose biodegradation by inoculation with lixivia (75%) relative humidity

Cellulose initiated state

Cellulose after 28 days, (slight growth)

Figure.7. Cellulose biodegradation by inoculation with lixivia (75%) relative humidity.

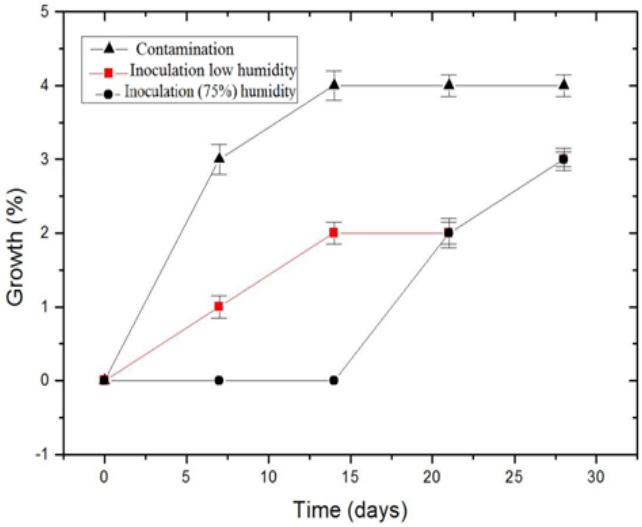
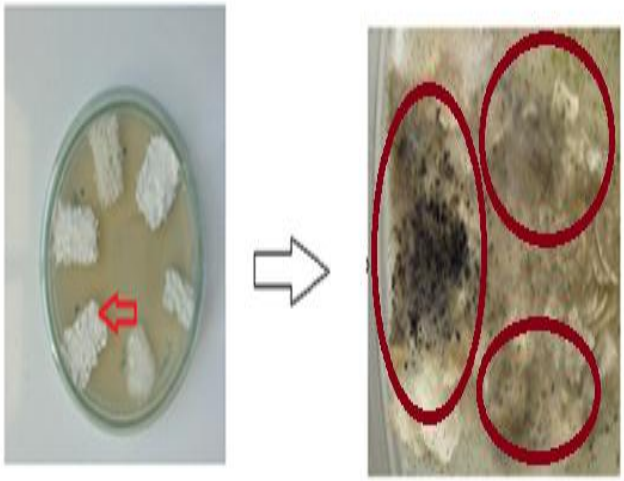


Figure.8. Percentage of microorganisms growth in surface of cellulose in different humidity conditions

The growth of microorganisms at the surface of cellulose is the faster in case of contamination at low humidity with a heavy growth after 28 days of incubation. The contamination by drops of lixivia at the surface of cellulose shows a plateau phase between 14 days and 21 days. We recorded a medium growth after 28 days of incubation and the plateau become larger between 1 day and 21 days. When the humidity increases we recorded a slight growth after 28 days of incubation.

3.3. Biodegradation of blend (A) : [cellulose (80%) + polybutylene succinate (20%)].

3.3.1. Biodegradation of blend (A) by contamination

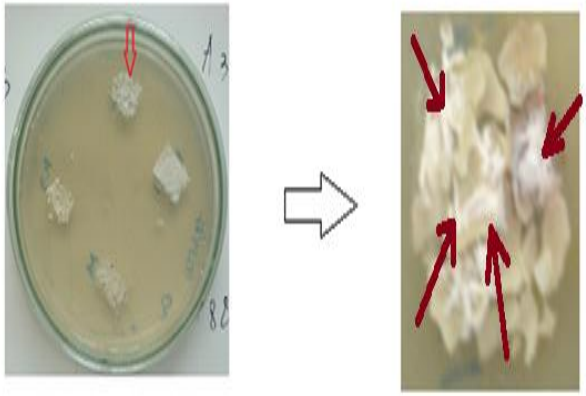


Blend initiated state

Blend after 28 days, (heavy growth)

Figure.9. Blend (A) biodegradation by contamination.

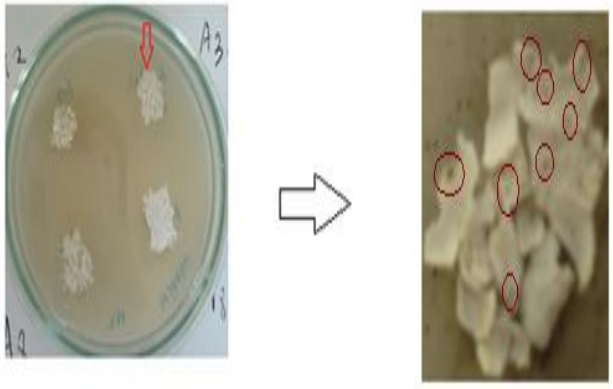
3.3.2. Blend (A) biodegradation by inoculation with lixivia low humidity



Blend initiated state — Blend after 28 days, (medium growth)

Figure.10. Blend (A) biodegradation by inoculation with lixivia low humidity

3.3.3. Blend (A) biodegradation by inoculation with lixivia at relative humidity (75%)



Blend initiated state — Blend after 28 days, (slight growth)

Figure.11. Blend (A) biodegradation by inoculation with lixivia at relative humidity (75%)

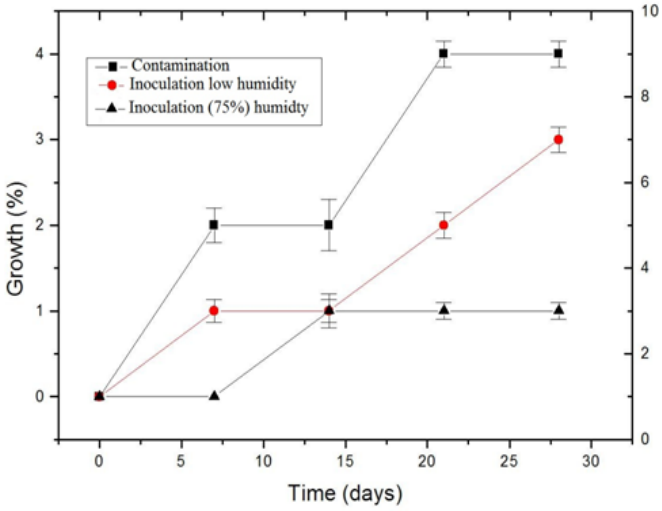
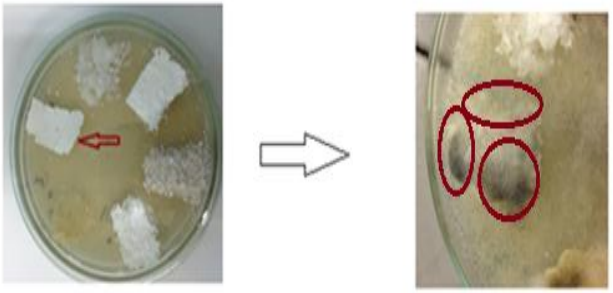


Figure.12. Microorganism's growth on the surface of blend (A) in different humidity conditions.

The microorganism's growth on the surface of blend (A) started faster in case of contamination; in low humidity we noted the presence of a plateau phase between 7 days and 14 days with a heavy growth after 28 days of incubation. The contamination by drops of lixivia in low humidity started faster but not so much and a plateau phase appeared between 7 days and 14 days and we recorded medium growth after 28 days of incubation. When the humidity increases, we noted a plateau phases apparition at the beginning between 1 day and 7 days and at the end of the process between 14 days and 28 days. We recorded a slight growth after 28 days of incubation.

3.4. Biodegradation of blend (B) : [cellulose (80%) + polycaprolactone (20%)].

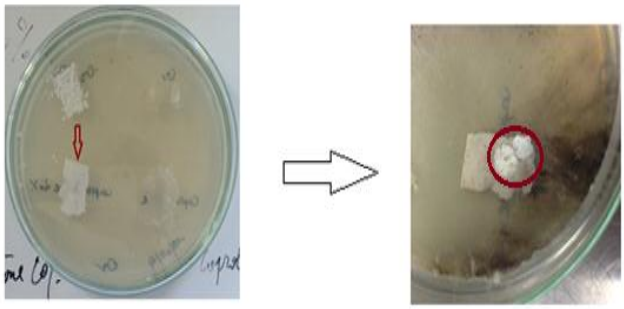
3.4.1. Biodegradation of blend (B) by contamination



Blend initiated state — Blend after 28 days, (heavy growth)

Figure.13. Blend (B) biodegradation by contamination

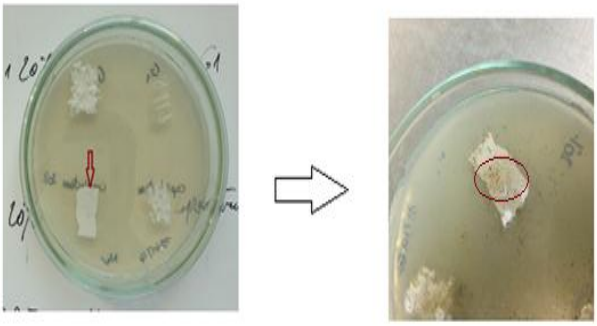
3.4.2. Blend (B) biodegradation by inoculation with lixivia at low humidity



Blend initiated state — Blend after 28 days, (medium growth)

Figure.14. Blend (B) biodegradation by inoculation with lixivia low humidity

3.4.3. Biodegradation of blend (B) by inoculation with lixivia at (75%) relative humidity.



Blend initiated state — Blend after 28 days, (slight growth)

Figure.15. Blend (B) biodegradation by inoculation with lixivia at relative humidity(75%).

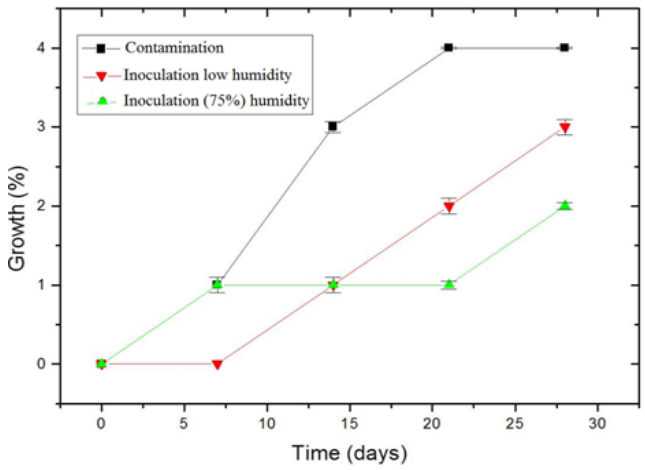


Figure.16. Microorganism's growth on the surface of blend (B) in different humidity conditions.

The microorganisms growth in the surface of blend [cellulose (80%) + polycaprolactone (20%)] faster in case of contamination in low humidity with a heavy growth after 28 days of incubation. The contamination by drops of lixivia in low humidity begins by a plateau phase in the range of 1-7 days and then started faster but not so much and we recorded a medium growth after 28 days of incubation. In the contamination by drops of lixivia in height humidity, the microorganism's growth starts faster in the surface of blend, a plateau phase is noted between 7 days and 21 days and after 28 days of incubation we recorded a slight growth.

Biodegradation is governed by different factors that include polymer characteristics, type of microorganisms, and nature of pretreatment. The polymer characteristics for example its mobility, tactility, crystallinity, all plays an important role in its degradation [26-27]. During biodegradation the polymer is first converted to its monomers, and then these monomers are mineralized. Most polymers are too large to pass through cellular membranes, so they must first be depolymerized to smaller monomers before they can be absorbed and biodegraded within microbial cells. At least two categories of enzymes are actively involved in biodegradation of polymers: extracellular and intracellular depolymerases [27]. During biodegradation, exo-enzymes from microorganisms break down complex polymers yielding smaller molecules of short chains.

Water can bind the OH group of a cellulose chain by hydrogen bonding or bridges hydroxyls from different chains. Direct bonding of an OH can occur either along the same cellulose chain or between different chains. Clusters of water molecules, especially at high water concentration [28-30], can form as well as crystalline and amorphous cellulose chain domains; even though there is no complete agreement yet about the water sitting and the role it plays in the ultra structure of cellulose [31].
Different sterilized samples (cellulose, blend (A) composed of (80%) of cellulose and (20%) of PBS and blend (B) composed of (80%) cellulose and (20%) of PCL are tested in aerobic biodegradation. Various parameters were followed. The growth percentage on the surface of samples (**Table1**), before and after natural contamination under low humidity, inoculation by drops of lixivia under low humidity and height humidity were monitored during 28 days.
The tests lead to the development of microorganisms capable to use our products (cellulose, Blend (A) and Blend (B)) as carbon source. It is noted visually that samples were largely colonized by the microorganisms under natural contamination in low humidity environment (cellulose (**Figure.5**), Blend (A) (**Figure.9**), Blend (B) (**Figure.13**), followed but not so much by samples inoculated by drops of lixivia in low humidity environment (cellulose (**Figure.6**), Blend (A) (**Figure.10**), Blend (B) (**Figure.14**). The samples inoculated by drops of lixivia in height humidity environment were smallest colonized by the microorganisms (cellulose (**Figure.7**), Blend (A) (**Figure.11**), Blend (B) (**Figure.15**)).

The visual following of microorganisms growth in surface of samples is faster in case of contamination in low humidity, but for blend (A) (cellulose (80%) + PBS (20%)) we recorded a plateau phase between 7 days and 15 days. Certainly Polybutylene succinate Provides excellent thermal and chemical stability [32, 33], but has a slow rate of biodegradation may be due to its high degree of crystalline [34], either polymer or copolymer [35-39], or a blend mixture with another polymer [40]. In the case of blend (B) (cellulose (80%) + PCL (20%)) no plateau phase was recorded. The semi crystalline PCL polymer is highly processible as it is has a slow melting point (55- 60 °C) and shows a glass transition temperature at 60 °C [41-45]. PCL is soluble in a wide range of organic solvents, giving the ability to form miscible blends with wide range of polymers. The semi crystalline PCL polymer offers more amorphous areas making the microorganism developments easer.

The inoculation by drops of lixivia at low humidity shows a growth percentage on the surface of samples which starts faster with a plateau phase for cellulose between the 14 day and the 21 day, for Blend (A) between the 7 day and the 15 day and for Blend (B) between first day and the 7 day. The growth percentage on the surface of samples by inoculating with drops of lixivia at high humidity showed that the plateau phases become larger and multiples, for cellulose between the first day and the 15 day, for Blend (A) between the first day and the 7 day and between the 15 day and the 21 day. Finally for Blend (B), the plateau phase is formed between the 7 day and the 21 day.

The plateau phases can be explained by of microorganisms adaptation. The growth synergic of many fungi can also cause small-scale swelling and bursting, as the fungi penetrate the polymer solids [46].When the humidity increases, the rapid initial uptake expresses the extreme hygroscopicity of dry cellulose, which reflects tight interactions between cellulose and water. It is generally admitted that the first water molecules adsorb on the polar accessible groups of cellulose as the amorphous areas, they are logically suspected to be the most favorable adsorption sites [47], thus the amorphous domains degrease and the crystalline domains show more difficulties for biodegradation phenomena. After an initial degradation, crystalline spherolites appear on the surface; that can be explained by a preferential biodegradation of the amorphous polymer fraction, etching the slower-degrading crystalline parts out of the material [48].

IV. Conclusions

In this study we have synthesized polybutylene succinate polymer. We have also created a blend between cellulose (80%) and polybutylene succinate (20%) and a blend between cellulose (80%) and a commercial polycaprolactone (20%). Under different environments we have study the biodegradation phenomena of cellulose and its composites, searching to understand the impact of water in the biodegradability acceleration rhythm.

According to this study, it was noted that cellulose and its blends (A) and (B) tested can be more or less potentially biodegradable, taking into account the conditions under which they are placed to incubate. In natural contamination and low humidity tests we can see that the surface of the solid phase is entirely colonized by micro-organisms, not so much for the inoculated by drops of lixivia, we noted a plateau phases .The plateau phases become larger and multiple when the humidity increases.

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