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Comparison of the Biodegradation of Polypropylene (PP) and Low Density Polyethylene (LDPE) by *Pseudomonas aeruginosa* and *Staphylococcus aureus* at Different pH Ranges under Mesophilic Condition

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The present study aims to assess the temporal variations of some biotic and abiotic biodegradation parameters of 2 polymers (LDPE and PP) by the bacteria *P. aeruginosa* and *S. aureus* at acidic, neutral, and alkaline pH under mesophilic conditions. LDPE and PP fragments were immersed in the mineral solutions free of carbon sources containing *P. aeruginosa* or *S. aureus* cells. The initial pH values (pHi) of solutions were 5, 7 and 9. Incubation was carried out at 28°C for 10, 20, 30, 40, 50 and 60 days. Results showed that during incubations, the pH of the solutions varies over time. In most cases, the electrical conductivity and cell abundances increased. The weights of the polymers decreased. The cell abundance apparent increasing rates (CAAIR), the electrical conductivity apparent increasing rates (ECAIR), and the polymer weight apparent decreasing rates (PWADR) were estimated. The highest PWADR in the presence of LDPE was 0.4 mg/10days with *P. aeruginosa*at pHi 5 and 7, and 0.6 mg/10days with *S. aureus* under pHi 7. For PP, it was constant (0.7 mg/10days) under all pHi with *S. aureus*, but decreased in the presence of *P. aeruginosa* with increasing in pHi solutions. The ECAIR with LDPE decreased with increasing pHi with both bacterial species.The highest ECAIR of PP was recorded under pHi 5 with *S. aureus* and under pHi 7 with *P. aeruginosa*.The highest CAAIR with LDPE was 185.18 CFU/10days for *P. aeruginosa* at pHi 5 and 116.79 CFU/10days for *S. aureus* at pHi 7. With PP, it was 297.61 CFU/10days for *P. aeruginosa* at pHi 5 and 67.64 CFU/10days for *S. aureus*at pHi 7. The biodegradation parameters values recorded with of *P. aeruginosa* differed significantly ($P<0.05$) from those recorded with *S. aureus* under each pHi.

Keywords: Biodegradation; bacteria species; initial pH medium; incubation duration; parameters assessment.

1. INTRODUCTION

Plastics are polymers made up of a wide range of organic and inorganic, synthetic or semi-synthetic compounds. They are made primarily from petrochemical materials extracted from coal, oil and natural gas [1]. The types of petroleum-derived polymers commonly used in the global economy to make single-use plastics include low density polyethylene (LDPE), high density polyethylene (HDPE), polypropylene (PP), polyvinyl chloride (PVC) and polyethylene terephthalate (PET). Polyethylene and PP are however the most abundant [2,3]. Due to their chemical and mechanical properties, these polymers exhibit adverse effects in the environment characterized by the accumulation of plastic waste in landfills and in aquatic environments as well as on the functioning of these ecosystems and the organisms that live there [4-6].

Managing plastic waste through the process of biodegradation would reduce the adverse effects of these plastics and it indeed allows an increase in environmental safety [7]. The biological elimination of these plastic polymers proceeds by the decomposition of the polymer. It requires the use of extracellular and intracellular depolymerases by organisms to break down organic substances [8].

Previous studies have shown the ability of several bacterial and fungal strains to degrade LDPE and PP [9,10]. It appears that this process, which can occur in fresh waters, seas and in the soil, can be the work of Gram-positive and Gram-negative fungi and bacteria. It is influenced by the chemical elements and the temerature of the environment. Few data are available on the impact of microbial biodegradation on the variations over time of the chemical characteristics of the environment. Little is known about the ease or difficulty of a specific microorganism in degrading this or that other polymer, as well as the difficulty or the ease for a specific polymer to be degraded by this or that other bacterium. The possibility of degradation of plastics that pollutes our environment by microorganisms lead to an increased interest towards the basic mechanism by which microorganism are able to degrade these polymers. The bacteria *Pseudomonas aeruginosa*, a Gram-negative bacillus and *Staphylococcus aureus*, Gram-positive cocci, are two ubiquitous, undemanding and very versatile microorganisms [11,12]. They also secrete many enzymes such as lipases and proteases, which are hydrolases that can be involved in many hydrolysis and oxidation reactions [13].

LDPE is a linear hydrocarbon polymer consisting of long chains of ethylene monomers (C_2H_4) and it is made from oil or gas extracts by efficient high pressure catalytic polymerization of ethylene monomers. Its density is about 0.920g/cm^{3}and the presence of branched chains is responsible for its low density [14]. Its properties include, among others, opacity, tear resistance, tensile strength, stiffness, chemical resistance and flexibility, even at low temperatures [14]. PP has the chemical formula (-CH2-CH(CH3)-)n and its density is about 0.9g/cm^3. It comes from the polymerization of propylene monomer and has many mechanical properties such as its high rigidity and impact resistance [15].

Few data are available on the potential of these two bacteria to degrade many plastic wastes in general, and LDPE and PP in particular. Little is known about the environmental conditions that can favor the degradation of LDPE and PP by these two bacteria. We also know little about the ease or difficulty for one or the other of the 2 polymers to be degraded by each of the 2 bacteria considered, or of the difficulty or ease of one or the other of the 2 bacteria to degrade one or the other of the 2 polymers. It is necessary to optimize the metabolism of microorganisms and specific environmental conditions for the degradation of specific pollutants in the environment, in general, and of LDPE and PP in aquatic systems in particular. The present study thus aims to evaluate the biodegradation of the 2 polymers by the bacteria *P. aeruginosa* and *S. aureus* in aquatic microcosm at acidic, neutral and alkaline pH under mesophilic temperature condition, and to determine the temporal variations of some biotic and abiotic biodegradation parameters.

2. MATERIALS AND METHODS

2.1 Making LDPE and PP Fragments

The 2 plastics considered are therefore of different densities [15]. For the experiments in the present study, we chose to make the polymers with the same equal surfaces. The PP and LDPE were thus cut into squares with sides of 4 cm. The weights were 0.1g for PP and 0.065g for LDPE. For each polymer the fragments were then immersed in a 70/30 (W/W%) water-ethanol mixture for sterilization according to Sandi [16], then removed using sterile forceps, dried for 4 hours at 45°C in an incubator and stored at room temperature (23 ± 1°C) in sterile Petri dishes.

The sterility of the polymers fragments was verified. For this, few pieces were put on a standard plate count agar poured into 15 Petri dishes 90 mm in diameter. These Petri dishes were then divided into 3 groups of 5 Petri dishes each. The first group was then incubated at 42°C for 2 days. The second was incubated at 37°C for 3 days, and the third was incubated at laboratory temperature (28 ± 1°C) for 5 days. The absence of any colony forming unit (CFU) after the various incubations testified the sterility of the plastic fragments stored.

2.2 Isolation and Identification of Bacterial Strains

The bacteria used in this study were *P. aeruginosa* and *S. aureus*. The 2 bacteria were isolated from surface water of Yaounde (Cameroon, Central Africa) using the membrane filtration method. For *P. aeruginosa*, the agar culture medium used was Cetrimide nalidixicacid agar (CN, Difco Laboratories, Detroit, MI, USA) contained in Petri dish. Incubation was done at 37°C for 24 hours. For *S. aureus*, the agar culture medium used was the Chapman agar(Mannitol salt MSA). Incubation was carried out at 37°C for 24-48 hours. The typical *P. aeruginosa* and *S. aureus* colonies were subsequently identified by using conventional biochemical tests [17,18].

2.3 Preparation of *P. aeruginosa* and *S. aureus* cultures

For the preparation of cell's stocks of each bacterial species, a colony forming unit (CFU) from CN agar medium for *P. aeruginosa*or from Chapman mannitol agar medium for *S. aureus*was inoculated into 100 mL of nutrient broth (Oxford) for 24 hours at 37°C. After this period, cells were harvested by centrifugation at 8000 rev/min for 10 min at 10 ºC and washed twice with NaCl (0.85%) solution. Each pellet was re-suspended in 50 mL of NaCl solution. After homogenization, 1mL of the obtained solution was then transferred into 500 mL of sterile NaCl solution (0.85%) in Erlenmeyer flask for later use.

2.4. Experimental Protocol

The biodegradability tests were carried out on incomplete media (free of carbon source) but containing mineral elements necessary for the bacteria. The mineral elements necessary for the

cells growth according to [16,18] included NH_4NO_3 (1g/L), KH_2PO_4 (0.7g/L), K_2HPO_4 (0.7g/L), $MgSO_4$, $7H_2O$ (0.7g/L), NaCl (0.005g/L), $FeSO_4$, $7H_2O$ (0.002g/L), $ZnSO_4$, $7H_2O$ (0.02g/L), $MnSO_4$, $7H_2O$ (0.001 g/L). They were thus dissolved in distilled water contained in Erlenmayer flasks of 250 mL, in the required weight concentration.

The experiments were carried out at 3 different initial pH (pHi) values for each bacterium and each polymer. These pHi values were pHi 5, pHi 7 and pHi 9. The pHi values were adjusted using a pH-meter, HCl (0.1N) and NaOH (0.1N) solutions. These glass flasks were then sterilized in the autoclave. After cooling the mineral suspensions, the sterile plastic fragments, prepared and stored as indicated above, were sterilely introduced into each vials. For each bacterium, each polymer and each pHi value, 21 glass flasks of 250 mL containing each 200 mL of the mineral solution were used. Then 2 mL of cells suspension compared to the Mc Farland solution previously prepared were introduced into each flask, and then homogenized. The cells concentration was thus adjusted to $1.6x10^7$ CFU/mL.

The 21 glass flasks were placed into 7 groups of 3 glass flasks each. Those groups were named d-0, d-10, d-20, d-30, d-40, d-50 and d-60. The triplets of d-10, d-20, d-30, d-40, d-50 and d-60 glass flasks were incubated under sterile conditions at 28 ± 2°C for 10 days, 20 days, 30 days, 40 days, 50 days and 60 days respectively.

2.5 Biodegradability Assessment of the LDPE and PP Fragments

The polymers biodegradability was assessed by determining the temporal changing of bacterial abundance, weight loss of the polymer fragments and chemical properties of the medium. At each initial moment (d-0), the weight of polymer fragment (using a balance), the pH value (using a pH-meter) and that of electrical conductivity (using a conductivity meter) of solutions, and the cells abundance were measured. At the end of each incubation period for each of the considered pHi value, the solutions in flasks were vigorously stirred. This allows the homogenization of the solution and the detachment of the bacterial cells adhered to the polymers fragments. After, the abundance of cultivable bacterial cells was assessed in each solution, and the pH and the electrical conductivity of the solution were measured. Each polymer fragment was then taken out of solution, and then dried and its weight determined.

Concerning the bacteriological analyses, after homogenization of the suspension in each bottle, the polymer fragment was removed under sterile conditions from the Erlenmeyer flask and introduced into a sterile 30 mL test tube to release the adhered bacterial cells. The unhooking of adherent cells was performed by vortex agitation at increasing speeds for 30 seconds in four consecutive series of 10 ml sterilized NaCl solution (8.5 g/l).This technique allows the unhooking of maximum adhered cells [19]. The total volume of the suspension containing the unhooked bacterial cells was 40 ml. A total volume of 40 mL of the cell detachment solution was thus added to the Erlenmeyer flask containing the stock biodegradation solution. After further homogenization, cell abundance was assessed by culture on selective agar media. As indicated above, CN agar medium was used for *P. aeruginosa* and Chapman mannitol agar for *S. aureus.* Petri dishes were then incubated during 24-48 hours at 37°C.

For each bacterium, each polymer and each initial pH (pHi) value, each of the 4 parameter was thus measured 3 times. The cell abundances were expressed in CFU/mL, the electrical conductivities in µS/cm, the weights of polymers in mg, and the pH in conventional units.

2.6. Data Analysis

The average of each parameter measured for each of the 3 initial pH conditions was determined, as each experiment was done 3 times. The temporal variation of studied parameters was illustrated using histogram.

An overall comparison of the values of each parameter recorded during the 7 incubation periods (d-0, d-10, d-20, d-30, d-40, d-50 and d-60) and for all 3 experimental pHi conditions of the solutions was carried out, for each bacterial species present and each polymer. This was carried out using the H-test of Kruskal-Wallis and SPSS programm. Then a comparison between the values of each parameter recorded during the 7 incubation periods in the presence of *P. aeruginosa* and those recorded in the presence of *S. aureus*, for each pHi condition was carried out for each polymer considered using the W-test of Wilcoxon and R programm.

The apparent rates of temporal variations in the weight of the plastic, the electrical conductivity and the cell abundances in the presence of each type of polymer and each bacterial species considered, and at each pHi condition of the solutions, were estimated. Here, the chosen incubation period unit was 10 days. For this, the regression lines showing the temporal variations of those parameters at each incubation period (d-0, d-10, d-20, d-30, d-40, d-50 and d-60) as a function of each experimental pHi condition were plotted. Each straight regression line equation (y = ax + b) was calculated using the least squares method [20,21]. In this equation, y is the dependent variable; x is the explanatory variable; a is the slope of the regression line, and b is the intercept point of the regression line with the y-axis (i.e. when x = 0). The slope of the regression line obtained under each experimental condition was assimilated to the apparent degradation rates of LDPE/PP fragments, to the increasing rate of the electrical conductivity of the solutions or to the changing rates in cell abundance. This changing rate in cell abundance was then compared to the cell apparent growth rate when positive, or to the cell apparent inhibition rate when negative [22]. Apparent rates of temporal changings of those measured parameters were assessed using the Excel Microsoft programm.

3. RESULTS

3.1 Temporal Variation of the pH Values of Solutions During Incubation

With LDPE, the pH values of the solutions varied during the incubations and this from one pHi to another, from one bacterial species to another (Fig. 1). Under pHi 5 and in the presence of *S. aureus*, a decrease in pH values was noted with increasing incubation duration. The lowest value 3.85 was recorded after 40 days. In the presence of *P. aeruginosa*, an increase in pH from 5 to 5.62 was first observed after 10 days, followed by a slight and gradual decrease during the remaining days of incubation. After 60 days, a value 5.12 was recorded (Fig. 1). Under pHi 7, the pH values decreased throughout the incubation period both in the presence of *P. aeruginosa* and *S. aureus*. The lowest values were 6.14 in the presence of *S. aureus* recorded after 60 days, and 6.86 in the presence of *P. aeruginosa* recorded after 10 days (Fig. 1). Under pHi 9, the pH value in the presence of *P. aeruginosa* decreased from 9 to 6.68 after 10 days, then oscillated around 6 during the remaining days of incubation. In the presence of *S. aureus*, the pH values varied throughout the incubation periods (Fig. 1).

With PP, temporal variations in pH values were also observed. Under pHi 5, the pH values of solutions in the presence *P. aeruginosa* first increased from 5 to 6 after 10 days. From the 20th day, a gradual decrease was observed. After 60 days, a value of 5.6 was recorded. In the presence of *S. aureus*, a gradual decrease in pH was observed with increasing incubation duration. The values recorded were 4.15, 4.14 and 4.02 respectively after 10 days, 40 days and 60 days (Fig. 1). The same observations as in the presence of LDPE were made for all pHi in the prsence of PP (Fig 1).

3.2 Temporal Variation of the Electrical Conductivity Values of Solutions During Incubation

With LDPE, a gradual increase in the electrical conductivity values of the solutions under pHi 5 and 7 was observed with increasing incubation duration. In the presence of *P. aeruginosa* they varied from 3198 to 4035 µs/cm under pHi 5 and from 3191 to 3785 µS/cm under pHi 7, after 60 days. In the presence of *S. aureus*, the electrical conductivity values varied from 3198 to 4150 µS/cm under pHi 5 and from 3191 to 4290 µS/cm under pHi 7, after 60 days (Fig. 2).Under pHi 9 and in the presence of *P. aeruginosa*, a gradual increase in electrical conductivity values was observed from 2943 µS/cm on beginning day to 3750 µS/cm after 40 days. In the presence of *S. aureus*, electrical conductivity values increased gradually throughout the incubation periods. The highest value was 3510 µS/cm, recorded after 60 days (Fig. 2).

With PP, the electrical conductivity also increased during the incubation periods and this under all pHi values. Under each pHi considered, its values in the presence of *S. aureus* were higher than those recorded in the presence of *P. aeruginosa* (Fig. 2). Under pHi 5, and in the presence of *P. aeruginosa* it fluctuated from 3065 µS/cm to 3250 µS/cm after 20 days, to 3785 µS/cm after 40 days and to 3560 µS/cm after 60 days. In the presence of *S. aureus*, the values were 3935, 4145 and 4340 µS/cm, respectively after 20 days, 40 days and 60 days (Fig. 2). Under pHi 7, a gradual increase in this parameter values was observed in the presence of *S. aureus* from 3182 µS/cm on the beginning day to 4730 µS/cm after 50 days. In the presence of *P.*

aeruginosa, the values increased from 3182 µS/cm to 3500 µS/cm after 10 days, to 3675 µS/cm after 30 days and to 3930 µS/cm after 60 days (Fig. 2).Under pHi 9, in the presence of *P. aeruginosa* and *S. aureus* it was respectively 3160 and 3405 µS/cm after 10 days, 3300 and 3520 µS/cm after 30 days and 3610 and 3630 µS/cm after 60 days (Fig. 2).

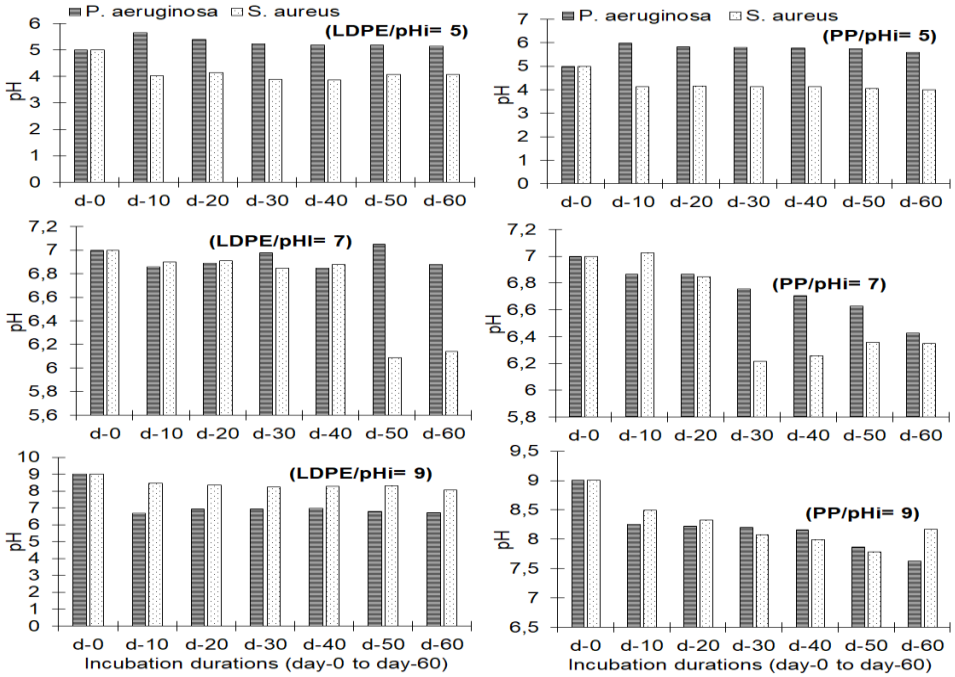


Fig. 1. Temporal variation of the pH of solutions containing the LDPE and PP fragments under each pHi condition and in the presence of *P. aeruginosa* and *S. aureus*

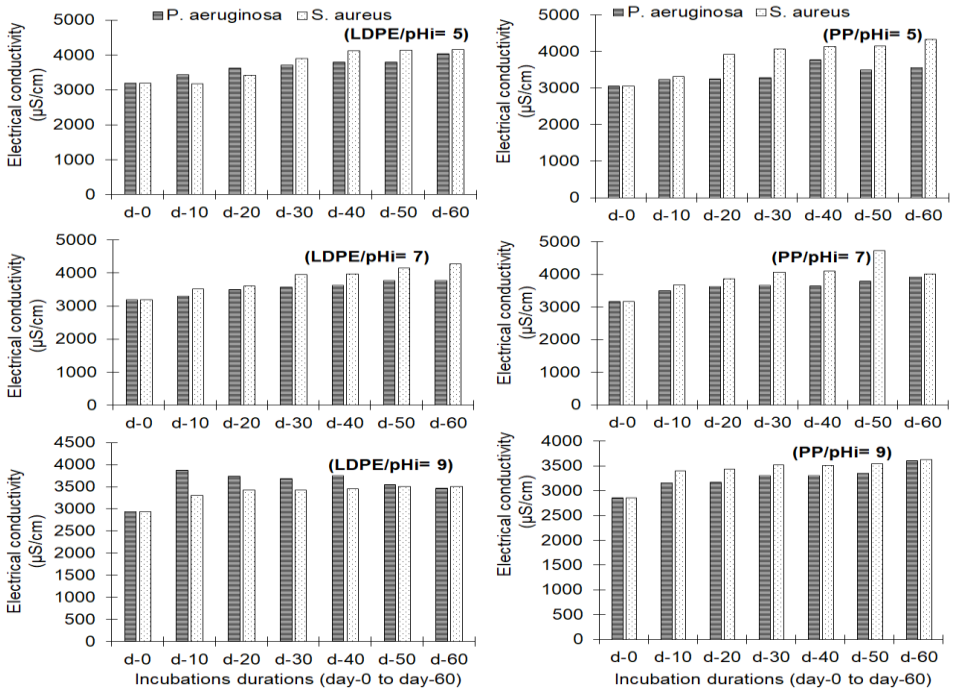


Fig. 2. Temporal variation of the electrical conductivity of solutions containing LDPE and PP fragments under each pHi condition and in the presence of *P. aeruginosa* and *S. aureus*

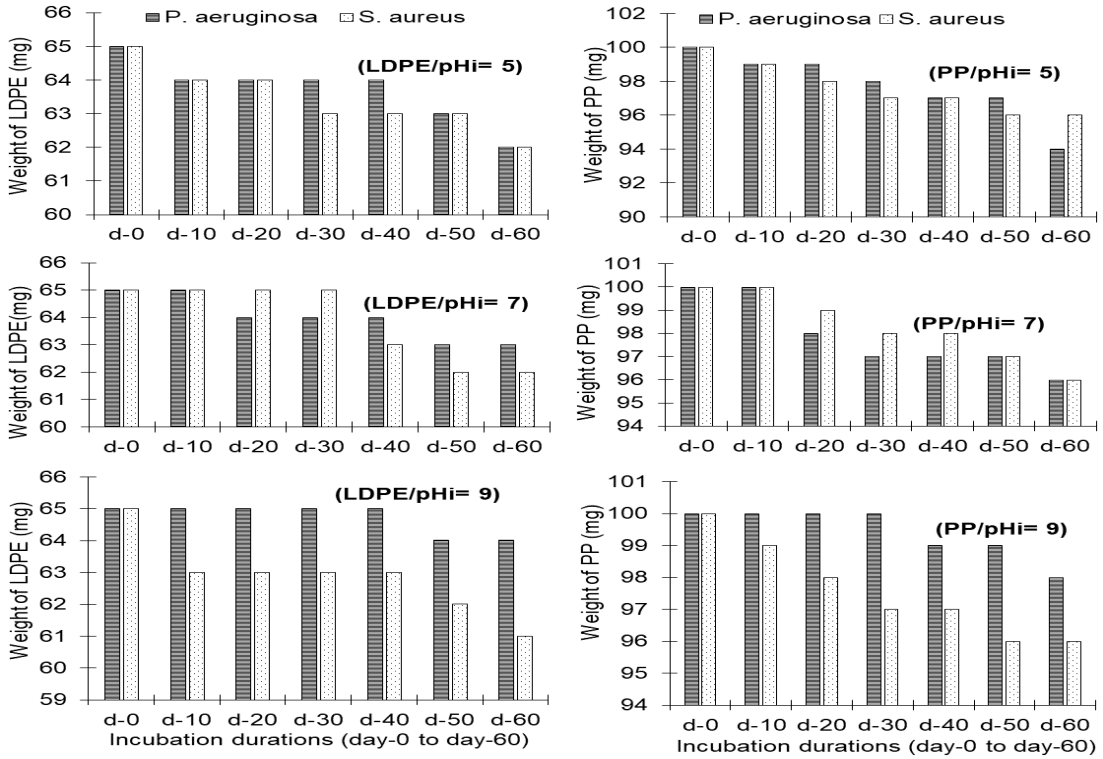


Fig. 3. Temporal variation of the weights of the LDPE and PP fragments under each pHi condition of solutions and in the presence of *P. aeruginosa* and *S. aureus*

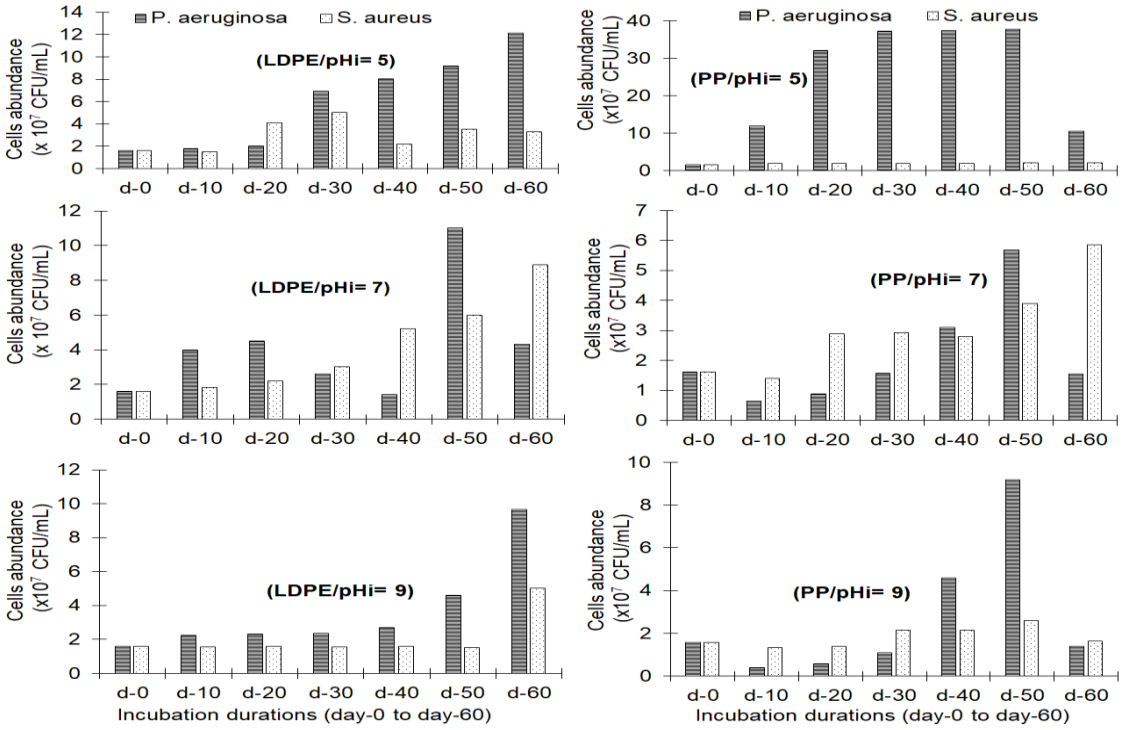


Fig. 4. Temporal variation of the of *P. aeruginosa* and *S. aureus* abundance in the presence of the LDPE and PP fragments and under each pHi condition of solutions

Table 1. P values of the overall comparison of the values of each parameter recorded during the 7 incubation periods and for all 3 experimental pHi conditions of the solutions

Type of polymer	Parameters considered and the bacterial species present in solution							
	Weight of fragments		pH		Electrical conductivity		Cells abundance	
	P.aerug	***S. aureus***	***P. aerug***	***S. aureus***	***P. aerug***	***S. aureus***	***P. aerug***	***S.aureus***
LDPE	P=0.031*	P=0.406	P=0.001**	P=0,.000**	P=0.662	P=0.099	P=0.813	P=0.035*
PP	P=0.006**	P=0.566	P=0.000**	P=0.000**	P=0.008**	P=0.003**	P=0.002**	P=0.000**

*P. aerug= Pseudomonas aeruginosa *: P<0.05 ; **: P< 0.01*

3.3 Temporal Variation of the Weights of Polymers in Solutions During Incubation

With LDPE as the polymer, a temporal fluctuation in the weight of the fragments was observed during the incubations, depending on the pHi condition of the solutions and the bacterial species present (Fig. 3). Under pHi 5, a gradual decrease of weight was observed from 65 mg to 64 mg after 10 days, to 63 mg after 50 days and to 62 mg after 60 days, both in the presence of *P. aeruginosa* and *S aureus*. Under pHi 7 and in the presence of *P. aeruginosa* a decrease in weight from 20th day was observed from 65 mg to 64 mg, then to 63 mg after 60 days. In the presence of the *S. aureus*, the weight did not vary as much after 10 days, 20 days and 30 days. After 40 days, a decrease was observed from 65 mg to 63 mg, then to 62 mg after 60 days (Fig. 3).Under pHi 9, the weight of LDPE in the presence of *P. aeruginosa* did not fluctuate during the first 40 days. After 50 days and 60 days, the weight decreased from 65 mg to 64 mg. However, in the presence of the *S. aureus*, a gradual decrease was noted with increasing incubation durations (Fig. 3).

With PP as polymer, the weight of the fragments also varied according to the pHi, the incubation periods and the bacterial species present (Fig. 3). Under pHi 5, the weight of the fragments in the presence of *P. aeruginosa* and *S. aureus* decreased with increasing incubation time. After 60 days, they fluctuated from 100 mg to 94 mg in the presence of *P. aeruginosa* and from 100 mg to 96 mg in the presence *of S. aureus*. Under pHi 7, the weight of PP did not change after 10 days in the presence of either *P. aeruginosa* or *S. aureus*. After 20 days, it decreased from 100 mg to 98 mg in the presence of *P. aeruginosa* and to 99 mg in the presence of *S. aureus*. After 30 days, 40 days, 50 days and 60 days, a gradual decrease was observed (Fig. 3).Under pHi 9 and in the presence of *P. aeruginosa*, the weight of PP did not vary after 10, 20 and 30 days. The lowest weight value, 98 mg, was recorded after 60 days. In the presence of *S. aureus*, a gradual decrease in the weight of PP was recorded with increasing incubation duration (Fig. 3).

3.4 Temporal Variation of the Cells Abundance in Solutions During Incubation

With LDPE, the cell abundances fluctuated from one pHi condition to another and according to the bacterial species considered (Fig. 4). Under pHi 5, the abundances of *P. aeruginosa* gradually increased with increasing incubation periods. Initially $1.6x10^7$ CFU/mL, the abundance recorded after 60 days was $12.1x10^7$ CFU/mL. Abundance of *S. aureus* first gradually increased from $1.6x10^7$ CFU/mL on the beginning day to $5x10^7$ CFU/mL after 30 days. Subsequently, a decrease to $2.2x10^7$ CFU/mL was observed after 40 days, and reached $3.5x10^7$ CFU/mL after 50 days and $3.3x10^7$ CFU/mL after 60 days (Fig. 4). Under pHi 7, abundance of *P. aeruginosa* also increased during the incubations. The highest abundance $11x10^7$ CFU/mL was recorded after 50 days. The lowest abundance, $1.4x10^7$ CFU/mL was recorded after 40 days. On the other hand, the abundances of *S. aureus* increased gradually with increasing incubation times. After 60 days, the abundance was $8.9x10^7$ CFU/mL (Fig. 4). Under pHi 9, the abundances of *P. aeruginosa* gradually increased. The highest abundance $9.64x10^7$ CFU/mL was recorded after 60 days. For *S. aureus* the highest cell abundance $5x10^7$ CFU/mL was recorded after 60 days (Fig. 4).

Table 2. **P values of the comparison for each polymer considered, between the values of each parameter recorded during the 7 incubation periods in the presence of *P. aeruginosa* and those recorded in the presence of *S. aureus*, under each pHi condition of the solutions**

Type of polymer and measured parameters		Conditions de pH initial (pHi) des solutions		
Type of polymer	**Parameters**	**pHi= 5**	**pHi= 7**	**pHi= 9**
LDPE	Polymer weight	P=0.436	P=1	P=0.009**
	pH	P=0.002**	P=0.336	P=0.025*
	Elec. Conductivity	P=0.710	P=0.249	P=0.037*
	Cells abundance	P=0.128	P= 0.455	P=0.017*
PP	Polymer weight	P=0.518	P=0.512	P=0.025*
	pH	P=0.002**	P= 0.405	P=0.847
	Elec. Conductivity	P=0.053	P=0.053	P=0.097
	Cells abundance	P=0.000**	P=0.710	P=0.017*

*Elec. Conductivity= electrical conductivity *: P<0.05 ; **: P< 0.01*

With PP, the cell abundances recorded during the incubations also fluctuated from one pHi to another and according to the bacterial species considered (Fig. 4). Under pHi 5, the abundances of *P. aeruginosa* were higher than those of *S. aureus* at all incubation periods. After 50 days, cell abundance was $37.9x10^7$ CFU/mL for *P. aeruginosa* and $2.2x10^7$ for *S. aureus*. After 60 days, a decrease in cell abundances was observed (Fig. 4). Under pHi 7, the *S. aureus* abundance was $1.4x10^7$ CFU/mL after 10 days. From the 20th day, a gradual increase was observed until the 60th day. The abundance of *S. aureus* was $5.84x10^7$ CFU/mL after 60 days. The abundance of *P. aeruginosa* also decreased from $1.6x10^7$ CFU/mL to $0.64x10^7$ CFU/mL after 10 days. From 20^{th} day to the 50th day, a gradual increase was observed and it reached $5.67x10^7$ CFU/mL (Fig. 4). Under pHi 9, cell abundances decreased from $1.6x10^7$ CFU/mL to $0.4x10^7$ for *P. aeruginosa* and from $1.6x10^7$ CFU/mL to $1.35x10^7$ CFU/mL for *S. aureus* after 10 days of incubation. From the 20th day, the abundances of the 2 bacteria increased (Fig. 4).

3.5 Overall Comparison of the Values of Each Parameter Recorded During the 7 Incubation Periods for all the 3 Experimental pHi Conditions of the Solutions and for Each Polymer and in the Presence of Each Bacterial Species

An overall comparison of the recorded values of each biodegradation parameter during the 7 incubation periods for all 3 experimental pHi conditions of the solutions was carried out using the Kruskal-Wallis H-test. The P values for each polymer considered and in the presence of each bacterial species are presented in Table 1

It is noted that during the degradation of LDPE, the weights variation during the 7 incubation periods differs significantly ($P<0.05$) from one pHi to another and in the presence of *P. aeruginosa*. But in the presence of *S. aureus*, no significant difference ($P> 0.05$) was observed in the variations of the weights fragments. The variations in pH of the biodegradation solutions recorded during the incubation periods were very significant ($P<0.01$) from one pHi to another, in the presence of *P. aeruginosa* and *S. aureus*. However, no significant difference was observed in the variations of electrical conductivity from one pHi to another in the presence of each of the 2 bacterial species. The abundances of *S. aureus*, varied significantly according to the pHi of the solutions, while those of *P. aeruginosa* did not during the degradation of LDPE (Table 1).

In the presence of PP fragments, the variations in the electrical conductivity, the pH of the solutions and the abundances of *P. aeruginosa* and *S. aureus* varied significantly ($P<0.01$) from one pHi to another, during incubations (Table 1). The weight of the PP fragments varied significantly ($P<0.05$) depending on the PHi of the solutions in the presence of *P. aeruginosa*. However, no significant difference was observed in the variation in the weight of the fragments of this polymer from one pHi to another in the presence of the bacterium *S. aureus* (Table 1).

3.6 Comparison between the Values of each Parameter Recorded During the 7 Incubation Periods in the Presence of *P. aeruginosa* and those Recorded in the Presence of *S. aureus* for Each Polymer Considered and Under Each pHi Condition of the Solutions

For each polymer, a comparison of the recorded values of the biodegradation parameters in the presence of the bacterium *P. aeruginosa* and those recorded in the presence of *S. aureus* and under each pHi, was carried out using the Wilcoxon W-test. P values are shown in Table 2.

With LDPE, the temporal variations in the weight of the fragments under pHi 5 and 7 in the presence of *P. aeruginosa* did not differed significantly ($P>0.05$) from those recorded in the presence of *S. aureus*. The difference was however very significant ($P<0.01$) when the pHi of the solutions was 9. The temporal variations of the pH of the solutions during the biodegradation of LDPE in the presence of *P. aeruginosa* differed significantly ($P<0.01$) of those observed in the presence of *S. aureus* when the pHi of the solutions is 5 or 9 (Table 2). The electrical conductivity values of the solutions differed significantly ($P<0.05$) between the presence of the 2 bacteria only when the pHi was 9. The same observation is made for the cell abundances ($P<0.05$) (Table 2).

With PP, the temporal variations in the weight of the fragments differed significantly ($P<0.01$) between the presence of the 2 bacterial species only when the pHi of the solutions was 9. The temporal variations in the pH of the solutions

Table 3. Values of PWADR, ECAIR and CAAIR (and regression coefficient r^2) during incubations, in the presence of each polymer and each bacterial species considered, and under each experimental pHi condition of solutions

Type of polymer and pHi value		Parameters considered and the bacterial species present in solution during					
		PWADR (mg/10days)		ECAIR (µS/cm/10 days)		CAAIR (CFU/10 days)	
Type of Polymer	**pHi value**	***P. aerug.***	***S. aureus***	***P. aerug.***	***S. aureus***	***P. aerug.***	***S. aureus***
LDPE	pHi= 5	-0.4 (r^2=0.796)	-0.4 (r^2=0.9)	122.36 (r^2=0.936)	195.21 (r^2=0.889)	185.18 (r^2=0.923)	24.10 (r^2=0.163)
	pHi= 7	-0.4 (r^2=0.892)	-0.6 (r^2=0.802)	102.39 (r^2=0.957)	175.96 (r^2=0.956)	65.82 (r^2=0.194)	116.79 (r^2=0.883)
	pHi= 9	-0.2 (r^2=0.625)	-0.5 (r^2=0.790)	32.89 (r^2=0.053)	75.92 (r^2=0.684)	102.61 (r^2=0.630)	34.39 (r^2=0.332)
PP	pHi= 5	-0.9 (r^2=0.878)	-0.7 (r^2=0.940)	91.42 (r^2=0.655)	203.39 (r^2=0.844)	297.61 (r^2=0.173)	4.53 (r^2=0.658)
	pHi= 7	-0.7 (r^2=0.867)	-0.7 (r^2=0.960)	102.11 (r^2=0.860)	171.57 (r^2=0.628)	48 (r^2=0.342)	67.64 (r^2=0.861)
	pHi= 9	-0.3 (r^2=0.778)	-0.7 (r^2=0.940)	99.28 (r^2=0.881)	95.53 (r^2=0.648)	79.07 (r^2=0.282)	16.25 (r^2=0.448)

P. aerug= Pseudomonas aeruginosa

between the presence of the 2 bacteria are significantly different (P<0.01) only when the pHi of the solutions was 5. For the electrical conductivity of the solutions, these temporal variations were not significantly different between the presence of the 2 bacteria (P>0.05). The variations in the abundances of *P. aeruginosa* during the biodegradation of PP differ significantly (P<0.05) from those of *S. aureus* when the pHi of the solutions is 5 or 9 (Table 2).

3.7 Assessment of the Parameters Changing Apparent Rates at the Presence of each Polymer and each Bacterial Species

It has been noted that during incubations, the pH of the solutions sometimes increases or decreases at the presence of each polymer and each bacterial species considered, and under each pHi condition of solutions. In most cases, the electrical conductivity increases, as well as the cell abundances. And the weights of the polymers decreased. The cells abundance apparent increasing rates (CAAIR), the electrical conductivity apparent increasing rates (ECAIR), and the polymers weight apparent decreasing rates (PWADR) were estimated per 10days by the linear regression model. The values of these apparent velocities are presented in Table 3.

It is noted that with LDPE fragments in the presence of the 2 bacteria *P. aeruginosa* and *S. aureus*, the PWADRs are identical under pHi 5 and are 0.4 mg/10days. These PWADRs decrease to 0.2 mg/10days under pHi 9 in the presence of *P. aeruginosa*, but increase to 0.6 mg/10days in the presence of *S. aureus* under pHi 7 of solutions (Table 3). With PP fragments in the presence of *P. aeruginosa* PWADR decrease from 0.9 mg/10days to 0.3 mg/10days with increasing pHi solution. On the other hand, in the presence of *S. aureus*, this PWADR was 0.7 mg/10days under all pHi values (Table 3).

The ECAIR of the solutions gradually decreased with increasing pHi of the solutions for both bacteria when the polymer was the LDPE. In the presence of *P. aeruginosa*, it increased from 122.36 µS/cm/10days under pHi 5 to 32.89 µS/cm/10 days under pHi 9 (Table 3). In the presence of *S. aureus*, it varied from 195.21 µS/cm/10 days (pHi 5) to 75.92 µS/cm/10days (pHi 9). With PP fragments in solutions, the ECAIR in the presence *of S. aureus* gradually decreased from 203.39 µS/cm/10days (pHi 5) to 95.53 µS/cm/10 days (pHi 9). In the presence of *P. aeruginosa*, the highest ECAIR 102.11 µS/cm/10days was recorded under pHi 7 (Table 3).

The highest CAAIR for *P. aeruginosa* with the LDPE fragments was 185.18 CFU/10days recorded under pHi 5. That of *S. aureus* was 116.79 CFU/10days recorded under pHi 7 (Table 3). The lowest CAAIRs were 65.82 CFU/10days for *P. aeruginosa* (pHi 7) and 24.10 CFU/10days for *S. aureus* (pHi 5) (Table 3). With the PP fragments, the highest CAAIR was 297.61 CFU/10days for *P. aeruginosa* (pHi 5) and 67.64 CFU/10days for *S. aureus* (pHi 7). The lowest were 48 CFU/10days for *P. aeruginosa* (pHi 7) and 4.53 CFU/10days for *S. aureus* (pHi 5) (Table 3).

4. DISCUSSION

This study showed that LDPE and PP are not completely inert towards micro-organisms, but have demonstrated certain, though limited long term biodegradability. Many microorganisms including bacteria have been known to possess polyester degrading mechanisms because of various enzymes found in these organisms. These enzymes include among others peroxidase, laccase, hydrolases, styrene monooxygenase, depolymerase, esterase, dehydrogenases, tannases, cutinases, lipases and carboxylesterases [2,23,24]. The microorganisms use plastics as their sole carbon source for their survival and hence can flourish on plastic waste when provided with optimal conditions necessary for growth. Decrease in molecular weight of the plastic monomer is one of the main criteria that is employed to testify whether plastic is being degraded or not in the presence of microorganisms [10,25].

The primary mechanism for the biodegradation of polymer is the oxidation or hydrolysis by enzyme to create functional groups that improves its hydrophilicity. Consequently, the main chains of polymer are degraded resulting in polymer of low molecular weight and feeble mechanical properties, thus, making it more accessible for further microbial assimilation [26].

It has been indicated that plastics considered in this study are exclusively of carbon atoms and not attached to reactive groups and lack hydrolyzable bonds that would allow hydrolytic degradation. For being non-hydrolyzable, their initial depolymerization relies on redox reactions that release oligomers of lower molecular weight. These may be utilized by microorganisms,

entering in diverse metabolic pathways [27,28]. Extracellular enzymes such as depolymerases and hydrolases act on large plastic polymers to break them down into smaller molecules. Hydrolytic cleavage can occur either at the polymer chain terminus (exo-attack) or somewhere along the polymer chain (endo-attack) [23]. The extracellular oxidase found in a number of *Pseudomonas* can oxidize the considered polymers into a diketone structure [23,29,30]. Changing in pH solution may affects polymers surface by changing the concentration of hydroxyl group radicals that could oxidize polymer surfaces [23]. This would partly explain the variations from one pHi to another, the values of the parameters measured.

Microorganisms that colonize the plastic surface first cause a reduction in the size of polymer, degrading it into monomers which can be absorbed into the microbial cells, and then these monomeric units are further acted upon inside their cells through enzymatic degradation, utilizing the monomers as carbon source for growth. On enzymatic degradation, mineralisation of the monomers takes place and end products that are given out include CO_2, H_2O, CH_4, N_2 and various other metabolic products [23,29]. This would lead the the increase in electrical conductivity of the aquatic environment as registered in this study. A further utilization of these end products can be very useful in eliminating harmful plastics completely from the environment. Li et al [31] working with *Pseudomonas putida* noted that the metabolism of ethylene glycol and its derivatives has resulted in different oxidation products such as glycolaldehyde, glyoxal, glycolate, and glyoxylate. All these products as indicated above would lead to the increase of the electrical conductivity of the medium as it has been noted in this study.

It has been noted that appearents changings rates of parameters varied according to the experimental conditions. It is known that important factors affecting the rate of biodeterioration include material composition, molecular weights, atomic composition and the chemical bonds in the structure, the physical and chemical characteristics of the surfaces, the indigenous microflora, and environmental conditions [26]. Degradation of many plastics begins with the attachment of microbes to its surface followed by the production of some extracellular enzymes. Under aerobic conditions, CO_2, water and microbial biomass are the final degradation products whereas in case of anaerobic/methanogenic condition CO_2, water, methane and microbial biomass are the end products. The conversion of the long chain polymer into CO_2 and water is a complex process. Its degradation makes it fragile and sensitive to further oxidation by enzymes secreted by the microorganisms [3].

The complete process of biodegradation has been divided into four stages [24]: a)-biodeterioration, which is the formation of carbonyl-groups by the action of oxidative enzymes released by microorganisms or induced by exterior agents; b)-biofragmentation, which involves hydrolysis and/or fragmentation of the polymer carbon chains and the release of intermediate products, mediated by enzymes secreted by microorganisms; c)-bioassimilation, whereby small hydrocarbon fragments released by biofragmentation are taken-up and metabolized by bacteria, and d)-mineralization, which is the transfer of hydrolysis products within the cell wall, intracellular conversion of hydrolysis products to microbial biomass with the associated release of carbon dioxide and water excreted out the cell. During this process, a transformation in its basic structure leads to the formation of oxidized oligomers, followed by bioassimilation of small cleavage fragments by the microorganisms [3,24,32]. This would explain the increase in abundance of bacterial cells observed.

The role of some major groups of enzymes involved in the degradation of plastic polymers has been determined by Hou and Majumder [33]. Thus, cytochrome P450 are oxidoreductases catalyzing the introduction of one atom of molecular oxygen into nonactivated C-H bonds. Monooxygenase are oxidoreductases incorporating one atom of the oxygen molecule into substrates. Aromatic ring hydroxylase are oxidoreductases incorporating two atoms of dioxygen into the aromatic ring with the dihydroxylation reaction. Esterase are hydroxylases splitting esters into an acid and an alcohol. And alpha/beta hydrolase are hydroxylases involved in diverse catalytic functions including hydrolysis, proteolysis, removal of a halogen atom, and others. These enzymes are found in many Proteobacteria and Firmicutes [33,34].

Biological plastic degradation occurs when microorganisms use their enzymatic apparatus to break down polymers into smaller molecules and

monomers. These may be used as carbon and energy sources and are ultimately mineralized by microorganisms, being converted into carbon dioxide, water, methane and other compounds [35,36]. Biological processes can usually be performed under various environmental conditions (including tempearture, pH, pressure), circumventing the utilization and production of dangerous chemicals and depending on the microorganisms concerned [36,37].

The biodegradation is widely accepted as selective, and biodegradable plastics usually break down upon interaction with UV, water, temperature, oxygen content, time, nutrient availability, enzymes, microorganisms' presence and gradual changes in pH [10,35,38]. Compiled information on microbial species and proteins associated with reports of plastic biodegradation, demonstrating that presumed plastic-degrading traits are widely dispersed across the microbial tree of life, althought it is not always possible to know if such a bacterium can only degrade such a plastic and not such another, because the same enzyme can be synthesized by several different bacterial species [36].

It is also indicated that in the natural environment, water, oxygen, UV and enzymes (via microorganisms) are among the main agents initiating the degradation of materials by hydrolysis or oxidation. The processes implemented at the molecular scale (chain cutting, modification of chemical groups, etc.), at the meso- and macroscopic scales, have very diverse consequences depending on the general properties of the material concerned (in particular the chemical structure, the nature of the constituent atoms, the molar mass of the polymer, the physical state of the polymer, the levels of stress suffered, among others), and on the other hand, environmental conditions (temperature, pH, rate of oxygen, exposure to light, among others) [39-41]. These 2 groups of factors would impact the relative kinetics of micro-biogeochemical mechanisms in the material in the natural environment.

5. CONCLUSION

Polypropylène (PP) and low density polyethylene (LDPE) in the presence of *P. aeruginosa* and *S. aureus* bacteria in an aquatic environment undergo biodegradation. During this process, the pH of the medium varies over time ; the rate of loss of polymer weight, and the rate of increase in cell abundance and the electrical conductivity of the medium vary on the one hand according to the initial pH value of the solution, and on the other hand according to the polymer and the bacteria present. The parameters values recorded in the presence of *P. aeruginosa* differed significantly ($P<0.05$) from those recorded in the presence of *S. aureus* under each initial pH of solutions. It seems necessary to take this factor into consideration during the microbial biodegradation processes of plastic waste.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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