Original Research Article

Biodegradation of polypropylene (PP) and lowdensitypolyethylene (LDPE) by *Pseudomonas aeruginosa* and *Staphylococcus aureus* in aquatic microcosms of different pH ranges under mesophilic condition: A comparative approach of some biotic and environmental parameters

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Abstract

The presentstudyaims to assess the temporal variations of somebiotic and abioticbiodegradationparameters of 2 polymers LDPE and PP by the bacteria*P. aeruginosa* and *S. aureus* in aquaticmicrocosm at acidic, neutral and alkaline pH undermesophilic condition. LDPE and PP fragments wereimmersed in the mineral solutions free of carbon source containing*P. aeruginosa* or *S. aureus*cells. The initial pH values (pHi) of solutions were 5, 7 and 9. Incubation wascarried out at 28°C for 10, 20, 30, 40, 50 and 60 days. It has been notedthatduring incubations, the pH of the solutions varies over time. In most cases, the electricalconductivity and cellabundancesincreased. The weights of the polymersdecreased. The cellsabundance apparent increasing rates (CAAIR), the electricalconductivity apparent increasing rates (ECAIR), and the polymersweight apparent decreasing rates (PWADR) wereestimated. The highest PWADR in the presence of LDPE was 0.4mg/10days with*P. aeruginosa*recordedunderpHi 5 and 7, and 0.6mg/10days with*S. aureus*recordedunderpHi 7. For PP, itwas constant (0.7mg/10days) under all pHiwith*S. aureus*, but decreased in the presence of *P. aeruginosa*from 0.9 to 0.3mg/10days withincreasing in pHi solutions. The ECAIR with LDPE decreasedwithincreasingpHi, from 122.36 to 32.89 µS/cm10days in the presence of *P. aeruginosa*, and from 195.21 to 75.92 µS/cm/10days in the presence of *S. aureus*. With PP, the highest ECAIR wasrecordedunderpHi 5 for *S. aureus* (203.39 µS/cm/10days) and underpHi 7 for *P. aeruginosa* (102.11 µS/cm/10days). The highest CAAIR with LDPE was 185.18 CFU/10days for *P. aeruginosa*recordedunder pHi5, and 116.79 CFU/10days for *S. aureus*recordedunderpHi 7. With PP, itwas 297.61 CFU/10days for *P. aeruginosa*recordedunderpHi 5 and 67.64 CFU/10days for *S. aureus*recordedunderpHi 7. The biodegradationparameters values recorded in the presence of *P. aeruginosa*differedsignificantly ($P<0.05$) fromthoserecorded in the presence of *S. aureus*undereachpHi solution. This factor seemsnecessary to takeintoconsiderationduring the microbialbiodegradationprocesses of plastic waste.

Key words: Biodegradation, bacteria species, LDPE, PP, initial pH medium, incubation duration, parameters assessment

1. Introduction

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Development of plastics expanded in the first 50 years of the twentieth century, with more than 10 classes of polymersbeingsynthesized. The success of plastics as a material has been substantial; they have proved versatile for use in a range of types and forms, includingnaturalpolymers, modifiednaturalpolymers, thermosetting plastics, thermoplastics and, more recently, biodegradable plastics [1].Plastics are polymers made up of a wide range of organic and inorganic, syntheticor semi-synthetic compounds [2]. They are made primarilyfrompetrochemicalmaterialsextractedfromcoal, oil and naturalgas[3]. The types of petroleum-derivedpolymerscommonlyused in the global economy to make single-use plastics includelowdensitypolyethylene (LDPE), high densitypolyethylene (HDPE), polypropylene (PP), polyvinylchloride (PVC) and polyethyleneterephthalate (PET). Polyethylene and PP are however the mostabundant[4,5]. Their use has grownexponentiallysince the 1950s, nowreaching 400 million tons per year[6]. However, their intensive use associatedwithpoor performance of waste management systems (includingtheir collection and capture at the end of their life), and losses and incivilities lead to theirtransfer and accumulation in the naturalenvironment[7].

The origins of plastics in the environment are diverse and varied. They can come from production industries, consumers or be a consequence of waste management [7,8]. Due to theirchemical and mechanicalproperties, thesepolymersexhibit adverse effects in the environmentcharacterized by the accumulation of plastic waste in landfills and in aquaticenvironments as well as on the functioning of theseecosystems and the organismsthat live there[9-11].

However, severalmethods have been developed over time to remedyenvironmental pollution by plastic waste. Theseincludeincineration, recycling, landfilling and leakage, and biologicaltreatment[12-14]. Incinerationproducesenergythat can be profitable if sold[12]. However, the large amounts of CO_2 and greenhousegasesmassivelyreleasedinto the atmosphere as a result of combustion processescontribute to global warming[15]. Recycling plastics helpsreduce the amount of plastic waste in the environment. However, recycled plastics sufferfromreducedmechanical performance [16-18]. The otherconsequence of this process isthatitoftenresults in losses of materials in the form of airbornemicroplastics and fibersthat can beresponsible for respiratoryinflammatoryreactions in humanswho inhale it[7,16]. Burying or dumping

bioplasticwaste in a landfillremains the least desirable option as itismarked by uncontrolleddegradation. This anaerobicdecomposition process produces fugitive methane, whichis a greenhousegaswhenit escapes from the recovery system [13]. Itswarmingpotentialis 25 to 36 times greaterthanthat of CO_2[13]. Leaksconcern plastic wastefound in aquatic or terrestrialenvironments and in public spaces[19]. Plastics containing additives, such as plasticizers and UV stabilizers, contaminate the soil and marine environment over time and impact the functioning of animal and cellular organs[20].

Managing plastic wastethrough the process of biodegradationwouldreduce the adverse effects of these plastics and itindeedallows an increase in environmentalsafety[21,22]. The biologicalelimination of these plastic polymersproceeds by the decomposition of the polymer. It requires the use of extracellular and intracellulardepolymerases by organisms to break down organic substances [23,24].

Synthetic plastics when mixed withotherwasteslowlydegrade in the environment. This resistancecouldbecircumvented by the intervention of bothenvironmental physico-chemicalfactors and microbialpotential[25]. In the environment, water, oxygen, UV and enzymes (via microorganisms) are among the main agents initiating the degradation of materials by hydrolysis or oxidation[7,26]. The processesimplemented at the molecularscale (chaincutting, modification of chemical groups, etc.), at the meso- and macroscopicscales, have very diverse consequencesdepending on the generalproperties of the materialconcerned (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, amongothers), and on the other hand, environmental conditions (temperature, pH, rate of oxygen, exposure to light, amongothers). These 2 groups of factors impact the relative kinetics of micro-biogeochemicalmechanisms in the material[7,26].

Previousstudies have shown the ability of severalbacterial and fungalstrains to degrade LDPE and PP [27-29]. It appearsthatthis 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 isinfluenced by the chemicalelements of the environment and isinfluenced by the temperature of the environment. Few data are available on the impact of microbialbiodegradation on the variations over time of the chemicalcharacteristics of the environment. Wealso know little about the ease or difficulty of a specificmicroorganism in degradingthis or thatotherpolymer, as well as the difficultyor the ease for a specificpolymer to bedegraded by this or thatotherbacterium. The possibility of degradation of plastics thatpollutesourenvironment by microorganisms lead to an increasedinteresttowards the basic mechanism by whichmicroorganism are able to degradethesepolymers. The

bacteria*Pseudomonas aeruginosa*, a Gram-negativebacillus and *Staphylococcus aureus*, Gram-positive cocci, are twoubiquitous, undemanding and very versatile microorganisms[30,31]. Theyalsosecretemany enzymes such as lipases and proteases, which are hydrolases that can beinvolved in manyhydrolysis and oxidationreactions[32,33].

LDPE is a linear hydrocarbon polymer consisting of long chains of ethylene monomers (C_2H_4) and it is made fromoil or gasextracts by efficient high pressure catalyticpolymerization of ethylenemonomers. Itsdensityis about 0.920g/cm^{3}and the presence of branchedchainsisresponsible for itslowdensity[34-36].Itspropertiesinclude, amongothers, opacity, tearresistance, tensilestrength, stiffness, chemicalresistance and flexibility, even at lowtemperatures[36]. PP has the chemical formula (-CH2-CH(CH3)-)n and itsdensityis about 0.9g/cm^3. It comesfrom the polymerization of propylenemonomerand has manymechanicalpropertiessuch as its high rigidity and impact resistance[37].

Few data are available on the potential of thesetwobacteria to degrademany plastic wastes in general, and LDPE and PP in particular. Little isknown about the environmental conditions that can favor the degradation of LDPE and PP by thesetwobacteria. Wealso know little about the ease or difficulty for one or the other of the 2 polymers to bedegraded by each of the 2 bacteriaconsidered, 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 isnecessary to optimize the metabolism of microorganisms and specificenvironmental conditions for the degradation of specificpollutants in the environment, in general, and of LDPE and PP in aquaticsystems in particular. The presentstudythusaims to evaluate the biodegradation of the 2 polymers by the bacteria*P. aeruginosa* and *S. aureus* in aquaticmicrocosm at acidic, neutral and alkaline pH undermesophilictemperature condition, and to determine the temporal variations of somebiotic and abioticbiodegradationparameters.

Comment [m3]: paraphrase all introduction

2. Materials and Methods

2.1. Making LDPE and PP fragments

The 2 plastics considered in thisstudy are therefore of differentdensities[35,37]. For the experiments in the presentstudy, we chose to make the polymerswith the sameequal surfaces. The PP and LDPE werethuscutinto squares withsides of 4 cm. The weightswere 0.1g for PP and 0.065g for LDPE. For eachpolymer the fragments were then immersed in a 70/30 (W/W%) water-ethanol mixture for sterilization according to [38], 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 (23 ± 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 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 nalidixic (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 Mannitol.Incubation wascarried out at 37°C for 24-48 hours. The typical *P. aeruginosa* and *S. aureus* colonies were subsequently identified by using conventional biochemical tests [39-41].

Comment [m4]: CetrimideNalidixicacid Agar

Comment [m5]: Chapman agar (Mannitol salt MSA)

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 [38,41] 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 experimentswerecarried out at 3 different initial pH (pHi) values for eachbacterium and eachpolymer. These pH (pHi) values werepHi 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 eachbacterium, eachpolymer and eachpHi 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 understerile 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

Several methods are used to assess the biodegradability of polymers fragments. These include, the formation of carbonyl group, the measurement of CO_2 production, the keto-carbonyl index, ester-carbonyl index, contac angle with water, surface energy, crystallinity, meltingtemperature, relative crystallinity, lamellarthickness, changes in the molecularweight, molecularweight distribution topography, tensilestrength, strainenergy, % elongation and extension, fieldemission scanning electronmicroscopy, fouriertransforminfraredspectroscopy, thermogravimetricanalysis, estimation of bacterial abundance, quantitative estimation of the weight loss of the polymer fragments, changes in the chemical properties of the medium amongst others [5,36,42,43]. In this study, biodegradability was assessed using the last 3 criteria.

Comment [m6]: Not necessary, please rephrase

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 conductivitymeter) 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 bacterialcellsadhered 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, afterhomogenization of the suspension in eachbottle, the fragment of the polymerwasremovedundersterile conditions from the Erlenmeyer flask and introducedinto a sterile 30 mL test tube to release the adheredbacterialcells. The unhooking of adherentcellswasperformed by vortex agitation at increasing speeds for 30 seconds in four consecutiveseries of 10 ml sterilizedNaCl solution (8.5 g/l).This technique allows the unhooking of maximum adheredcells[44]. The total volume of the suspension containing the unhookedbacterialcellswas 40 ml. A total volume of 40 mL of the celldetachment solution wasthusadded to the Erlenmeyer flaskcontaining the stock biodegradation solution. Afterfurtherhomogenization, cellabundancewasassessed by culture on selective agar media.As indicatedabove, CN agar medium was used for *P. aeruginosa*and Chapman mannitol agar for *S. aureus*.Petri disheswerethenincubatedduring 24-48 hoursat 37°C.

For eachbacterium, eachpolymer and each initial pH (pHi) value, each of the 4 parameterwasthusmeasured 3 times. The cellabundanceswereexpressed in CFU/mL, the electricalconductivities in µS/cm, the weights of polymers in mg, and the pH in conventionalunits.

2.6. Data analysis

The average of eachparametermeasured for each of the 3 initial pH conditions wasdetermined, as eachexperimentwasdone 3 times. The temporal variation of studied parameters was illustrated using histogram.

An overallcomparison of the values of eachparameterrecordedduring the 7 incubation periods (d-0, d-10, d-20, d-30, d-40, d-50 and d-60) and for all 3 experimentalpHi conditions of the solutions wascarried out, for eachbacterialspeciespresent and eachpolymer. This was carried out using the H-test of Kruskal-Wallis and SPSS programm.Then a comparisonbetween the values of eachparameterrecordedduring the 7 incubation periods in the presence of *P. aeruginosa* and thoserecorded in the presence of *S. aureus*, for eachpHi condition was~~also~~carried out for eachpolymerconsideredusing the W-test of Wilcoxon and R programm.

The apparent rates of temporal variations in the weight of the plastic, the electricalconductivity and the cellabundances in the presence of each type of polymer and eachbacterialspeciesconsidered, and at eachpHi condition of the solutions, wereestimated. 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 method of least squares [45-47]. 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 rates in cell abundance was then compared to the cell apparent growth rate when positive, or to the cell apparent inhibition rate when negative [48]. Apparent rates of temporal changings of thosemeasuredparameterswereassessedusing 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 variedduring the incubations and thisfrom one pHi to another, from one bacterialspecies to another (Fig. 1). Under pHi 5 and in the presence of *S. aureus*, a decrease in pH values wasnotedwithincreasing incubation duration. The lowest value 3.85 wasrecordedafter 40 days. In the presence of *P. aeruginosa*, an increase in pH from 5 to 5.62 was first observedafter 10 days, followed by a slight and gradualdecreaseduring the remainingdays of incubation. After 60 days, a value 5.12 wasrecorded (Fig. 1). Under pHi 7, the pH values decreasedthroughout the incubation periodboth in the presence of *P. aeruginosa* and *S. aureus*. The lowest values were 6.14 in the presence of *S. aureus*recordedafter 60 days, and 6.86 in the presence of *P. aeruginosa*recordedafter 10 days (Fig. 1). Under pHi 9, the pH value in the presence of *P. aeruginosa*decreasedfrom 9 to 6.68 after 10 days, thenoscillatedaround 6 during the remainingdays of incubation. In the presence of *S. aureus*, the pH values decreasedthroughout the incubation period. Theywere 8.47 after 10 days, 8.24 after 30 days and 8.04 after 60 days (Fig. 1).

With PP, temporal variations in pH values werealsoobserved. Under pHi 5, the pH values of solutions in the presence*P. aeruginosa* first increasedfrom 5 to 6 after 10 days. From the 20th day, a gradualdecrease wasobserved. After 60 days, a value of 5.6 wasrecorded. In the presence of *S. aureus*, a gradualdecrease in pH wasobservedwithincreasing incubation duration. The values recordedwere 4.15, 4.14 and 4.02 respectivelyafter 10 days, 40 days and 60 days (Fig. 1). Under pHi 7 condition, the pH values of the solutions generallydecreasedthroughout the incubation periods. In the presence of *P. aeruginosa*, theyfluctuatedfrom 7 to 6.87 after 10 days, to 6.71 after 40 days and to 6.43 after 60 days. In the presence of *S. aureus*, the pH value first increasedslightlyfrom 7 to 7.03 after 10 days, thendecreased to 6.26 after 40 days and was 6.35 after 60 days (Fig. 1). Under pHi 9, pH of solutions alsodecreasedoverall. After 10 days, 30 days, 50

days and 60 days, itwasrespectively 8.25, 8.2, 7.87 and 7.63 in the presence*of P. aeruginosa*, and 8.49, 8.07, 7.78 and 8.17 in the presence of *S. aureus* (Fig. 1).

3.2. Temporal variation of the electrical conductivity values of solutions during incubation

With LDPE, a gradualincrease in the electricalconductivity values of the solutions underpHi 5 and 7 wasobservedwithincreasing incubation duration. In the presence of *P. aeruginosa*theyvariedfrom 3198 to 4035 µs/cm underpHi 5 and from 3191 to 3785 µS/cm underpHi 7, after 60 days. In the presence of *S. aureus*, the electricalconductivity values variedfrom 3198 to 4150 µS/cm underpHi 5 and from 3191 to 4290 µS/cm underpHi 7, after 60 days (Fig. 2).Under pHi 9 and in the presence of *P. aeruginosa*, a gradualincrease in electricalconductivity values wasobservedfrom 2943 µS/cm on beginningday to 3750 µS/cm after 40 days. The value decreasedslightlyafter 50 days and 60 days. In the presence of *S. aureus*, electricalconductivity values increasedgraduallythroughout the incubation periods. The highest value was 3510 µS/cm, recordedafter 60 days (Fig. 2).

With PP, the electricalconductivityalsoincreasedduring the incubation periods and thisunder all pHi values. Under eachpHiconsidered, its values in the presence of *S. aureus*werehigherthanthoserecorded in the presence of *P. aeruginosa* (Fig. 2). Under pHi 5, and in the presence of *P. aeruginosa*itfluctuatedfrom 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, respectivelyafter 20 days, 40 days and 60 days (Fig. 2).Under pHi 7, a gradualincrease in thisparameter values wasobserved in the presence of *S. aureus*from 3182 µS/cm on the beginningday to 4730 µS/cm after 50 days. At the 60th day, the electricalconductivitydecreased to 4015 µS/cm. In the presence of *P. aeruginosa*, the values increasedfrom 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*itwasrespectively 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).

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 wasobservedduring the incubations, depending on the pHi condition of the solutions and the bacterialspeciespresent (Fig. 3). Under pHi 5, a gradualdecrease of weightwasobservedfrom 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* the weightdid not change after 10 days. From 20th day, a decrease in weightwasobservedfrom 65 mg to 64 mg, then to 63 mg after 60 days. In the presence of the *S. aureus*, the weightdid not vary as muchafter 10 days, 20 days and 30 days. After 40 days, a decreasewasobservedfrom 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 fluctuateduring the first 40 days. After 50 days and 60 days, the weightdecreasedfrom 65 mg to 64 mg. However, in the presence of the *S. aureus*, a gradualdecreasewasnotedwithincreasing incubation durations. The weightwas 63 mg after 20 days, 62 mg after 50 days and 61 mg after 60 days (Fig. 3).

With PP as polymer, the weight of the fragments alsovariedaccording to the pHi, the incubation periods and the bacterialspeciespresent (Fig. 3). Under pHi 5, the weight of the fragments in the presence of *P. aeruginosa* and *S. aureus*decreasedwithincreasing incubation time. After 60 days, theyfluctuatedfrom 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, itdecreasedfrom 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 gradualdecreasewasobserved up to 96 mg in the presence of the 2 bacterialspecies (Fig. 3).Under pHi 9 and in the presence of *P. aeruginosa*, the weight of PP did not varyafter 10, 20 and 30 days. The lowestweight value, 98 mg, wasrecordedafter 60 days. In the presence of *S. aureus*, a gradualdecrease in the weight of PP wasrecordedwithincreasing incubation time. After 60 days, the weight of PP was 96 mg (Fig. 3).

3.4. Temporal variation of the cells abundance in solutions during incubation

With LDPE, the cellabundancesfluctuatedfrom one pHi condition to another and according to the bacterialspeciesconsidered (Fig. 4). Under pHi 5, the abundances of *P. aeruginosa*graduallyincreasedwithincreasing incubation periods. Initially 1.6 x 10^7 CFU/mL, the abundancerecordedafter 60 dayswas 12.1 x 10^7 CFU/mL. Abundance of *S. aureus* first graduallyincreasedfrom 1.6 x 10^7 CFU/mL on the beginningday to 5 x 10^7 CFU/mLafter 30 days. Subsequently, a decrease to 2.2 x 10^7 CFU/mLwasobservedafter 40 days. It wasfollowed by an increase. The cellabundancereached 3.5 x 10^7 CFU/mLafter 50 days and 3.3 x 10^7 CFU/mLafter 60 days (Fig. 4).Under pHi 7, abundance of *P. aeruginosa*alsoincreasedduring the incubations. The temporal variation profile of the *P. aeruginosa*abundancesseemssinusoidal. The highestabundance 11x10^7 CFU/mLwasrecordedafter 50 days. The lowestabundance, 1.4x10^7 CFU/mLwasrecordedafter 40 days. On the other hand, the abundances of *S.*

*aureus*increasedgraduallywithincreasing incubation times. After 60 days, the abundancewas 8.9 x 10^7 CFU/mL (Fig. 4).Under pHi 9, the abundances of *P. aeruginosa*graduallyincreased. The highestabundance 9.64x10^7 CFU/mLwasrecordedafter 60 days. For *S. aureus* the highestcellabundance 5 x 10^7 CFU/mLwasrecordedafter 60 days (Fig. 4).

With PP, the cellabundancesrecordedduring the incubations alsofluctuatedfrom one pHi to another and according to the bacterialspeciesconsidered (Fig. 4). Under pHi 5, the abundances of *P. aeruginosa*werehigherthanthose of *S. aureus* at all incubation periods. After 50 days, cellabundancewas 37.9 x 10^7 CFU/mL for *P. aeruginosa* and 2.2 x 10^7 for *S. aureus*. After 60 days, a decrease in cellabundanceswasobserved. It was 10.6 x 10_7 for *P. aeruginosa* and 2.12 x 10^7 CFU/mL for *S. aureus* (Fig. 4).Under pHi 7, the *S. aureus*abundancewas 1.4 x 10^7 CFU/mLafter 10 days. From the 20th day, a gradualincreasewasobserveduntil the 60th day. The abundance of *S. aureus*was 5.84 x 10^7 CFU/mLafter 60 days. The abundance of *P. aeruginosa*alsodecreasedfrom 1.6 x 10^7 CFU/mL to 0.64 x 10^7 CFU/mLafter 10 days. From 20^{th}day to the 50th day, a gradualincreasewasobserved and itreached 5.67 x 10^7 CFU/mL. However, on the 60th day, a decrease in abundancewasrecorded. It wasthus 1.55 x 10^7 CFU/mL (Fig. 4).Under pHi 9, cellabundancesdecreasedfrom 1.6 x 10^7 CFU/mL to 0.4 x 107 for *P. aeruginosa* and from 1.6 x 107 CFU/mL to 1.35 x 10^7 CFU/mL for *S. aureus*after 10 days of incubation. From the 20th day, the abundances of the 2 bacteriaincreaseduntil the 50th day, and thentheyfellagainafter 60 days. The highestcellabundance values wererecorded at the 50th day and were 9.2 x 10^7 CFU/mL for *P. aeruginosa* and 2.62 x 10^7 CFU/mL for *S. aureus*(Fig. 4).

3.5. Overallcomparison of the values of eachparameterrecordedduring the 7 incubation periods for all the 3 experimentalpHi conditions of the solutions and for eachpolymer and in the presence of eachbacterialspecies

An overallcomparison of the recorded values of eachbiodegradationparameterduring the 7 incubation periods for all 3 experimentalpHi conditions of the solutions wascarried out using the Kruskal-Wallis H-test. The P values for eachpolymerconsidered and in the presence of eachbacterialspecies are presented in Table 1

It isnotedthatduring the degradation of LDPE, the weights variation during the 7 incubation periodsdifferssignificantly ($P<0.05$) from one pHi to another and in the presence of *P. aeruginosa*. But in the presence of *S. aureus*, no significantdifference ($P> 0.05$) wasobserved in the variations of the weights fragments. The variations in pH of the biodegradation solutions recordedduring the incubation periodswereverysignificant ($P<0.01$) from one pHi to another, in the presence of *P.*

aeruginosa and *S. aureus*. However, no significantdifferencewasobserved in the variations of electricalconductivityfrom one pHi to another in the presence of each of the 2 bacterialspecies. The abundances of *S. aureus*, variedsignificantlyaccording to the pHi of the solutions, whilethose of *P. aeruginosa*did notduring the degradation of LDPE (Table 1).

In the presence of PP fragments, the variations in the electricalconductivity, the pH of the solutions and the abundances of *P. aeruginosa* and *S. aureus*variedsignificantly ($P<0.01$) from one pHi to another, during incubations (Table 1). The weight of the PP fragments variedsignificantly($P<0.05$) depending on the PHi of the solutions in the presence of *P. aeruginosa*. However, no significantdifferencewasobserved in the variation in the weight of the fragments of thispolymerfrom one pHi to another in the presence of the bacterium*S. aureus* (Table 1).

3.6. Comparisonbetween the values of eachparameterrecordedduring the 7 incubation periods in the presence of *P. aeruginosa* and thoserecorded in the presence of *S. aureus* for eachpolymerconsidered and undereachpHi condition of the solutions

For eachpolymer, a comparison of the recorded values of the biodegradationparameters in the presence of the bacterium P. aeruginosa and thoserecorded in the presence of S. aureus and undereachpHi, wascarried out using the Wilcoxon W-test. P values are shown in Table 2.

With LDPE, the temporal variations in the weight of the fragments underpHi 5 and 7 in the presence of *P. aeruginosa*did not differedsignificantly ($P>0.05$) fromthoserecorded in the presence of *S. aureus*. The differencewashoweververysignificant ($P<0.01$) 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*differedsignificantly ($P<0.01$) of thoseobserved in the presence of *S. aureus*when the pHi of the solutions is 5 or 9 (Table 2). The electricalconductivity values of the solutions differedsignificantly ($P<0.05$) between the presence of the 2 bacteriaonlywhen the pHiwas 9. The same observation is made for the cellabundances ($P<0.05$) (Table 2).

With PP, the temporal variations in the weight of the fragments differedsignificantly ($P<0.01$) between the presence of the 2 bacterialspeciesonlywhen the pHi of the solutions was 9. The temporal variations in the pH of the solutions between the presence of the 2 bacteria are significantlydifferent ($P<0.01$) onlywhen the pHi of the solutions was 5. For the electricalconductivity of the solutions, these temporal variations were not significantlydifferentbetween the presence of the 2 bacteria ($P>0.05$). The variations in the abundances of *P. aeruginosa*during the biodegradation of PP differsignificantly ($P<0.05$) fromthose 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 notedthatduring incubations, the pH of the solutions sometimesincreases or decreasesat the presence of each polymer and each bacterial species considered, and under each pHi condition of solutions. In most cases, the electricalconductivityincreases, as well as the cellabundances. And the weights of the polymersdecreased. The cellsabundance apparent increasing rates (CAAIR), the electricalconductivity apparent increasing rates (ECAIR), and the polymersweight apparent decreasing rates (PWADR) wereestimatedper 10days by the linearregression model. The values of these apparent velocities are presented in Table 3.

It isnotedthatwith LDPE fragments in the presence of the 2 bacteria*P. aeruginosa* and *S. aureus*, the PWADRs are identicalunderpHi 5 and are 0.4 mg/10 days. ThesePWADRsdecrease to 0.2 mg/10 daysunderpHi 9 in the presence of *P. aeruginosa*, but increase to 0.6 mg/10 days in the presence of *S. aureus*underpHi 7 of solutions (Table 3). With PP fragments in the presence of *P. aeruginosa*PWADRdecreasefrom 0.9 mg/10 days to 0.3 mg/10 dayswithincreasingpHi solution. On the other hand, in the presence of *S. aureus*, thisPWADRwas 0.7 mg/10 daysunder all pHi values (Table 3).

The ECAIR of the solutions graduallydecreasedwithincreasingpHi of the solutions for bothbacteriawhen the polymerwas the LDPE. In the presence of *P. aeruginosa*, itincreasedfrom 122.36 µS/cm/10 daysunderpHi 5 to 32.89 µS/cm/10 daysunderpHi 9 (Table 3). In the presence of *S. aureus*, itvariedfrom 195.21 µS/cm/10 days (pHi 5) to 75.92 µS/cm/10 days (pHi 9). With PP fragments in solutions, the ECAIR in the presence*of S. aureus*graduallydecreasedfrom 203.39 µS/cm/10 days (pHi 5) to 95.53 µS/cm/10 days (pHi 9). In the presence of *P. aeruginosa*, the highestECAIR 102.11 µS/cm/10 dayswasrecordedunderpHi 7 (Table 3).

The highestCAAIR for *P. aeruginosa*with the LDPE fragments was 185.18 CFU/10 daysrecordedunderpHi 5. That of *S. aureus*was 116.79 CFU/10 daysrecordedunderpHi 7 (Table 3). The lowestCAAIRswere 65.82 CFU/10 days for *P. aeruginosa* (pHi 7) and 24.10 CFU/10 days for *S. aureus* (pHi 5) (Table 3). With the PP fragments, the highestCAAIRwas 297.61 CFU/10 days for *P. aeruginosa* (pHi 5) and 67.64 CFU/10 days for *S. aureus* (pHi 7). The lowestwere 48 CFU/10 days for *P. aeruginosa* (pHi 7) and 4.53 CFU/10 days for *S. aureus* (pHi 5) (Table 3).

4. Discussion

This studyshowedthat LDPE and PP are not completelyinerttowardsmicro-organisms, but have demonstrated certain, thoughlimited long termbiodegradability.

Manymicroorganismsincluding fungi and bacteria have been known to possess polyester degradingmechanismsbecause of various enzymes found in theseorganisms. These enzymes include among others peroxidase, laccase, hydrolases, styrene monooxygenase, depolymerase, esterase,dehydrogenases, tannases, cutinases, lipases and carboxylesterases [4,49,50]. The microorganisms use plastics as their sole carbon source for theirsurvival and hence can flourish on plastic wastewhenprovidedwith optimal conditions necessary for growth. Decrease in molecularweight of the plastic monomeris one of the main criteriathatisemployed to testifywhether plastic isbeingdegraded or not in the presence of microorganisms[29,51].

The primarymechanism for the biodegradation of polymeris the oxidation or hydrolysis by enzyme to createfunctional groups thatimprovesitshydrophilicity. Consequently, the main chains of polymer are degradedresulting in polymer of lowmolecularweight and feeblemechanicalproperties, thus, makingit more accessible for furthermicrobial assimilation [52].

It has been indicatedthat plastics considered in thisstudy are exclusively of carbonatoms and not attached to reactive groups and lackhydrolyzable bonds thatwouldallowhydrolyticdegradation. For being non-hydrolyzable, their initial depolymerization relies on redox reactionsthat release oligomers of lowermolecularweight. Thesemaybeutilized by microorganisms, entering in diverse metabolicpathways [53,54]. Extracellular enzymes such as depolymerases and hydrolases act on large plastic polymers to break them down intosmallermolecules[55]. Hydrolyticcleavage can occureither at the polymerchain terminus (exo-attack) or somewherealong the polymerchain (endo-attack) [49]. The extracellularoxidasefound in a number of *Pseudomonas* can oxidize the consideredpolymersinto a diketone structure [49,56,57]. Changing in pH solution may affects polymers surface by changing the concentration of hydroxyl group radicalsthatcouldoxidizepolymer surfaces [49]. This wouldpartlyexplain the variations from one pHi to another, the values of the parametersmeasured

The hydrolases involved in the cleavage of plastic polymer lead to cleavage of long carbonchains in a twostep process. All the plastics that are present in the environment are hydrophobic in nature. The extracellular enzymes that are produced by variousmicroorganismsfirstlyadhere to the plastic surface throughhydrophobic interactions in the first step of enzyme-polymer interaction. Hydrophobiccleftpresent in many hydrolases near the active site of the enzyme can accommodate the hydrophobic groups present in the polymertherebyincreasing the accessibility of the enzyme to the polymer[49,51]. In the second stage of the reaction, the active site of the enzyme participates in the hydrolyticcleavage of the long polymerchainsintosmallermonomers or dimerswhich can beaccumulated by the microbialorganism and consumed as a carbon source [51,58].

Microorganismsthatcolonize the plastic surface first cause a reduction in the size of polymer, degradingitintomonomerswhich can beabsorbedinto the microbialcells, and thenthesemonomericunits are furtheracteduponinsidetheircellsthroughenzymaticdegradation, utilizing the monomers as carbon source for growth. Enzymaticdegradation of plastic enzymes are biocatalystswhichparticipate in a reaction, act on a particularsubstrate, and accelerate the process of conversion of thatsubstrateinto a valuableproduct. This process can befurthermodifiedif the plastics are treatedprior to microbialattack to break down the polymerintomonomericunitsthroughchemical or physicalmethodsincludingheating, cooling, freezing, thawing and chemicaldegradation[43,51]. On enzymaticdegradation, mineralisation of the monomerstakes place and end productsthat are given out include CO_2, H_2O, CH_4, N_2 and variousothermetabolicproducts. This would lead the theincrease in electricalconductivity of the aquaticenvironment as registered in thisstudy. A furtherutilization of these end products can beveryuseful in eliminatingharmful plastics completelyfrom the environment[59] 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 notedthatappearentschangings rates of parametersvariedaccording to the experimental conditions. It isknownthat important factorsaffecting the rate of biodeteriorationincludematerial composition, molecularweights, atomic composition and the chemical bonds in the structure, the physical and chemicalcharacteristics of the surfaces, the indigenousmicroflora, and environmental conditions [52,60].

Degradation of many plastics begins with the attachment of microbes to its surface followed by the production of some extracellular enzymes [34]. 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 [34,35]. 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 [5].

The complete process of biodegradation has been divided into four stages [50]: 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 [5,50,61]. 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 [62]. Thus,cytochrome P450 are oxidoreductasescatalyzing the introduction of one atom of molecularoxygenintononactivated C-H bonds. Monooxygenase are oxidoreductasesincorporating one atom of the oxygenmoleculeintosubstrates. Aromatic ring hydroxylase are oxidoreductasesincorporatingtwoatoms of dioxygeninto the aromatic ring with the dihydroxylationreaction. Esterase are are hydroxylases splitting esters into an acid and an alcohol. And alpha/beta hydrolase are hydroxylases involved in diverse catalyticfunctionsincludinghydrolysis, proteolysis, removal of a halogenatom, and others. These enzymes are found in manyProteobacteria and Firmicutes [62].

To date, variousmicrobial plastic-degrading enzymes have been discovered, representingpromisingbiocatalyst candidates for plastic depolymerization. The plastic-degrading enzymes identifiedso far mightonlyaccount for a small portion of the enzymes relevant to plastic depolymerization in the environment[63].

Biological plastic degradationoccurswhenmicroorganisms use theirenzymaticapparatus to break down polymersintosmallermolecules and monomers. Thesemaybeused as carbon and energy sources and are ultimatelymineralized by microorganisms, beingconvertedintocarbondioxide, water, methane and other compounds [64,65]. Biologicalprocesses can usuallybeperformedundervariousenvironmental conditions (includingtempearture, pH, pressure), circumventing the utilization and production of dangerouschemicals and depending on the microorganismsconcerned[65,66].

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 [12,29,64].

Compiled information on microbialspecies and proteinsassociatedwith reports of plastic biodegradation, demonstratingthatpresumed plastic-degrading traits are widelydispersedacross the microbialtree of life, althoughtitis not always possible to know if such a bacterium can

onlydegradesuch a plastic and not suchanother, because the same enzyme can besynthesized by severaldifferentbacterialspecies[65].

5. Conclusion

PP and LDPE in the presence of *P. aeruginosa* and *S. aureus*bacteria in an aquaticenvironmentundergobiodegradation. This process isaccompanied by a loss of polymerweight, an increase in cellabundance and the electricalconductivity of the medium. The pH of the medium varies over time. The rates of temporal change of thesevariousparametersvary on the one hand according to the initial pH value of the solution, and on the other hand according to the polymer and the bacteriapresent. The biodegradationparameters values recorded in the presence of *P. aeruginosa*differedsignificantly ($P<0.05$) fromthoserecorded in the presence of *S. aureus*undereach initial pH values solution. This factor seemsnecessary to takeintoconsideration in the process of microbialbiodegradation of plastic waste.

6. References

[1] AndradyAL, Neal MA. Applications and societal benefits of plastics.Philos Trans R Soc Lond B Biol Sci.2009; 364:1977-1984. Doi:10.1098/rstb.2008.0304

[2] Saminathan P, Sripriya A, Nalini K, Sivakumar T, ThangapandianVBiodegradation of plastics by *Pseudomonas putida* isolated from garden soil samples. J Advanced Botany and Zoology. 2014;1(3):34-38

[3] Temoor A, Muhammad S, Farrukh A, Ijaz R, Asad Ali S, Muhammad N, Amir H, Natasha M, Irfan M, Sher M. Biodegradation of plastics: current scenario and future prospects for environmental safety. Environ Sci Pollution Res. 2018. Doi:10.1007/s11356-018-1234-9

[4] Danso D, Chow J, Streit WR. Plastics: Environmental and biotechnological perspectives on microbialdegradation. Appl Environ Microbiol. 2019;85:e01095-19. Doi:10.1128/AEM.01095-19

[5] Montazer Z, HabibiNajafi MB, Levin DB. Challenges with verifying microbial degradation of polyethylene. Polymers.2020;12:123. Doi:10.3390/polym12010123

[6] Plastics Europe. Plastics-the facts: une analyse des données européennes sur la production, la demande et les déchets de plastiques. PlasticsEurope. 2018, Bruxelles, Belgique.

[7] Galgani F, Bruzaud S, Duflos G, Fabre P, Casdaldi E, Ghiglione J, Grimaud R, Matthieu G, Huvet A, Lagarde F, Ika P-P, Ter Halle A. Pollution des océans par les plastiques et les microplastiques. Techniques de l'Ingénieur. 2020. Archimer, BIO9300, 17 P. https://archimer.ifremer.fr/doc/00663/77471/

[8] Nerland IL, Halsband C, Allan I, Thomas KV. Microplastiques dans les environnements marins : occurrence, distribution et effets (Re.no.6754-2014). Institut norvégien de recherche sur l'eau, Oslo, 2014; pp 1-71. http://www.miljodirektoratet.no/Documents/publikasjoner/M319/M319.pdf. (Accessed 30th december 2018)

[9] Carlton JT, Chapman JW, Geller JB, Miller JA, Carlton DA, McCuller M, Trenaman NA, Steve BP, Ruiz GM. Tsunami-driven rafting: transoceanic species dispersal and implications for marine biogeography. Science. 2017;357: 1402-1406. Doi:10.1126/science.aao1498

[10] Koch D, Mihalyi B. Assessing the Change in Environmental Impact Categories when Replacing Conventional Plastic with Bioplastic in Chosen Application Fields. Chemical Engeneering Transactions. 2018;70:853-858.Doi:10.3303/CET1870143

[11] Lagana P, Caruso G, Corsi I, Bergami E, Venuti V, Majolino D, La Ferla R, Azzaro M. Do plastics serve as a possible vector for the spread of antibiotic resistance? Int J Hygiene Environ Health. 2019;222(1):89-100. Doi:10.1016/j.ijheh.2018.08.009

[12] Folino A, Karageorgiou A, Calabro PS, Komilis D. Biodegradation of wasted bioplastics in natural and industrial environments: A review.Sustainability. 2020;12:6030. Doi:10.3390/su12156030

[13] Hottle TA, Bilec MM, Landis AE. Biopolymer production and end of life comparisons using life cycle assessment. Resources Conservation and Recycling. 2017;122:295-306. Doi:10.1016/j.resconrec.2017.03.002

[14] Meereboer KW, Misra M, Mohanty AK. Review of recent advances in the biodegradability of polyhydroxyalkanoate (PHA) bioplastics and their composites. Green Chemistry. 2020; 22:5519-5558. Doi:10.1039/D0GC01647K.

[15] Gomez EF, Michel FC. Biodegradability of conventional and bio-based plastics and natural fiber composites during composting, anaerobic digestion and long-term soil incubation. Polymer Degradation and Stability. 2013; 98:2583-2591. Doi:10.1016/j.polymdegradstab.2013.09.018

[16] Badia JD, Gil-Castell O, Ribes-Greus A. Long-term properties and end-of-life of polymers from renewable resources. Polymer Degradation and Stability. 2017;137:35-57. Doi:10.1016/j.polymdegradstab.2017.01.002

[17] Singh N, Hui D, Singh R, Ahuja IPS, Feo L, Fraternali F. Recycling of plastic solid waste: A state of art review and future applications. Composites Part B Engineering. 2017;115:409-422. Doi:10.1016/j.compositesb.2016.09.013

[18] Vu DH, Akesson D, Taherzadeh MJ, Ferreira JA. Recycling strategies for polyhydroxyalkanoate-based waste materials: An overview. BioresourceTechnology. 2020; 298 :1-9. Doi:10.1016/j.biortech.2019.122393

[19] Cierjacks A, Behr F, Kowarik I. Operational performance indicators for litter management at festivals in semi-natural landscapes. Ecological Indicators. 2012;13:328-337. Doi:10.1016/j.ecolind.2011.06.033

[20] Gallo F, Fossi C, Weber R, Santillo D, Sousa J, Ingram I, Nadal A, Romano D. Marine litter plastics and their toxic chemicals components: the need for urgent preventive measures. Environmental Sciences Europe. 2018;30(13):1-14. Doi:10.1186/s12302-018-0139-z

[21] Ali MI, Ahmed S, Robson G, Javed I, Ali N, Atiq N, Hameed A. Isolation and molecular characterization of polyvinyl chloride (PVC) plastic degrading fungal isolates. J BasicMicrobiol. 2014; 54:18-27. Doi:10.1002/jobm.201200496

[22] Butbunchu N, Pathom-Aree W. Actinobacteria as promising candidate for polylactic acid type bioplastic degradation. Frontiers in Microbiology. 2019;10:1-10. Doi:10.3389/fmicb.2019.02834

[23] Dela Torre DYZ, Delos Santos LA, Reyes MLC, Baculi RQ. Biodegradation of low-density polyethylene by bacteria isolated from serpentinization-driven alkaline spring. Philippine ScienceLetters. 2018;11:1-12.

[24]Kolvenbach BA, Helbling DE, Kohler HE and Corvini PFX. Emerging chemicals and the evolution of biodegradation capacities and pathways in bacteria**.** Curr Opinion Biotechnol. 2014;27: 8-14.Doi:10.1016/j.copbio.2013.08.017

[25] Devi RS, Kannan VR, Natarajan K, Nivas D, Kannan K, Chandru S, Antony AR. The role of microbes in plastic degradation. Environ Waste Manage ed. Chandra, R. 2016 pp. 341-370 United States: CRC Press. Doi:10.1201/b19243-13

[26] Andrady A. The plastic in Microplastics, Marine Pollution Bulletin. 2017;119(1):12-22. Doi: 10.1016/j.marpolbul.2017.01.082

[27] Mehmood CT, Qazi IA, Hashmi I, Bhargava S, Deepa S. Biodegradation of low density polyethylene (LDPE) modified with dye sensitized titania and starch blend using *Stenotrophomonas pavanii*. Int Biodeterioration Biodegradation. 2016;113:276-286. Doi: 10.1016/j.ibiod.2016.01.025

[28] Santacoloma-Londono S, Buitrago-Gonzalez ME, Lamus-Molina V, Asprilla S, Ruíz-Teran JE, Villegas-Méndez LC. Evaluation of the biodegradation of polyethylene, polystyrene and polypropylene, through controlled tests in solid suspension with the fungus *Aspergillus flavus***.** Scientia et Technica Año. 2019; 24(03):532-540.

[29] Mouafo Tamnou EB, Tamsa Arfao A, Nougang ME, Metsopkeng CS, Noah Ewoti OV, Moungang LM, Nana PA, Atem Takang-Etta L-R, Perrière F, Sime-Ngando T,Nola M. Biodegradation of polyethylene by the bacterium *Pseudomonas aeruginosa* in acidic aquatic microcosm and effect of the environmental temperature. Environmental Challenges. 2021;3, 100056 Doi:10.1016/j.envc.2021.100056

[30] Accarias S. Impact du phénotype des macrophages résidents sur la nature de la réponse inflammatoire précoce lors d'une infection par *Staphylococcus aureus*, Thèse de Doctorat, Université de Toulouse. 2014 ; 212 P.

[31] Cottalorda A. Diversité phénotypique et moléculaire d'isolats urinaires de *Pseudomonas aeruginosa*. Médecine humaine et pathologie. Thèse de Doctorat, Normandie Université, 2020.

[32] Lays C. ARN régulateurs de *staphylococcus aureus* : rôle de RsaA dans la formation du biofilm et de la capsule, niveaux d'expression des ARN dans les prélèvements cliniques. Thèse de doctorat, sciences agricoles. Université Claude Bernard-LyonI. 2012;215 P.

[33] Novotny E, Erbanov P, Sezimov H, Malachov KR, Rybkov Z, Malinov L, Prokopov I, Brozek J. Biodegradation of aromatic-aliphatic copolyesters and polyesteramides by esterase activity-producing microorganisms. Int Biodeterioration Biodegradation.2015;97:25-30. Doi:10.1016/j.ibiod.2014.10.010

[34] Arutchelvi J, Sudhakar M, Arkatkar A, Doble M, Bhaduri S, Uppara P. Biodegradation of polyethylene and polypropylene. Indian J Biotechnol. 2008;7: 9-22.

[35] Sangale MK, Shahnawaz M, Ade AB. A review on biodegradation of polythene: the microbial approach. J BioremedBiodegrad. 2012 ;3:64. Doi:10.4172/2155-6199.1000164

[36] Ghatge S, Yang Y, Ahn J-H and Hur H-G. Biodegradation of polyethylene: a brief Review. Appl Biol Chem. 2020;63:27. Doi:10.1186/s13765-020-00511-3

[37] Duan H, Chen W, Meng H, Jianfang L, Gu K, Li J. Effects of photoaging on the tribological properties of engineering plastics: Tribological properties of engineering plastics. Polymer Advanced Technologies. 2014;25(9): 975- 980. Doi:10.1002/pat.3337

[38] Saadi Z. Etude de la dégradation fongique des polymères : cinétique de dégradation des polymères et caractérisation des sous-produits de dégradation - Etude de l'écotoxicité de ces polymères. Thèse de Doctorat, Universite Du Maine -UFR-Sciences et Technologie. 2008;151 P

[39] Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST. Bergey's Manual of Determinative Bacteriology. Lippincott Williams and Wilkins, Philadelphia, PA. 2000.

[40] Gyung Yoon M, Jeong Jeon H, Nam Kim M. Biodegradation of polyethylene by a soil bacterium and AlkB cloned recombinant cell. J BioremedBiodegrad. 2012;3(4):1000144. Doi:10.4172/2155-6199.1000145

[41] Su SSS, Lae KZW, Ngwe H. Isolation and identification of *Pseudomonas aeruginosa* from clinical soil. University of Yangon Res J. 2018;8, 271-275. Doi:10.33899/edusj.1970.163328

[42] Min K, Cuiffi JD, Mathers RT. Ranking environmental degradation trends of plastic marine debris based on physical properties and molecular structure. Nature Communications. 2020;11:727. Doi:10.1038/s41467-020-14538-z.

[43] Shazia Khan, Sharique A, Ayesha SA.Biodegradation of low density polyethylene (LDPE) by mesophilic fungus '*Penicillium citrinum*' isolated from soils of plastic waste dump yard, Bhopal, India.Environ Technol. 2022. Doi:10.1080/09593330.2022.2027025

[44] LontsiDjimeli C, TamsaArfao A, RossiV, Nsulem N, Raspal V,Bricheux G, Nola M, Sime-Ngando T. Impact of two disinfectants on detachment of *Enterococcus faecalis* from polythene in aquatic microcosm.Rese Biotechnol**.** 2016;7:28-42. Doi:10.19071/rib.2016.v7.2981

[45] Bailey NTJ. Statistical methods in biology. Hodder and Stoughton ed, London.1981.

[46] Tofallis C. Least squares percentage regression. J Modern. Applied Statistical Methods 2008;7(2): 526-534. Doi:10.22237/jmasm/1225513020

[47] Popovic B. The optimal solution to the problems by regression analysis. J EngManag Competitiveness. 2019;9(1):25-37. Doi:10.5937/JEMC1901025P

[48] Nola M, Eheth JS, Nougang ME, Moungang ML, Noah Ewoti OV, Krier F, Chihib N-E, Servais P, Hornez J-P, Njiné T. Assessment of *in-situ* abundance dynamics of enterobacteria

and total heterotrophic aerobic bacteria in groundwater in the equatorial region of Central Africa. Water SA 2012;38(5):737-746. Doi:10.4314/wsa.v37i4.18

[49] Wilkes RA, Aristilde L. Degradation and metabolism of synthetic plastics and associated products by *Pseudomonas* sp.: capabilities and challenges. J Appl Microbiol. 2017. Doi:10.1111/jam.13472

[50]Mohanan N, Montazer ZK, Sharma P, Levin DB. Microbial and enzymatic degradation of synthetic plastics. Frontiers in Microbiology 11. 2020; Article 580709. Doi:10.3389/fmicb.2020.580709

[51] Kaushal J, Khatri M, Arya SK. Recent insight into enzymatic degradation of plastics prevalent in the environment: A mini – review. Cleaner Engin Technol. 2021;2, 100083. Doi:10.1016/j.clet.2021.100083

[52] Sana Sheik, Chandrashekar KR, Swaroop K, Somashekarappa HM. Biodegradation of gamma irradiated low density polyethylene and polypropylene by endophytic fungi. Int BiodeterBiodegr. 2015;105:21-29. Doi:10.1016/j.ibiod.2015.08.006

[53] Krueger MC, Harms H, Schlosser D. Prospects for microbiological solutions to environmental pollution with plastics. Appl MicrobiolBiotechnol. 2015;99:8857-8874. Doi: 10.1007/s00253-015-6879-4

[54] Barcoto MO, Rodrigues A. Lessons From Insect Fungiculture: From Microbial Ecology to Plastics Degradation. Frontiers in Microbiology. 2022;13:812143. Doi:10.3389/fmicb.2022.812143

[55] Shah AA, Hasan F, Hameed A, Ahmed S. Biological degradation of plastics: a comprehensive review. Biotechnology Advances. 2008;26(3):246-265. Doi:10.1016/j.biotechadv.2007.12.005

[56] Shah Z, Hasan F, Krumholz L, Atkas D and Shah AA. Degradation of polyester polyurethane by newly isolated *Pseudomonas aeruginosa* strain MZA-85 and analysis of degradation products by GC-MS. Int BiodeteriorBiodegrad. 2013;77:114-122. Doi:10.1016/j.ibiod.2012.11.009

[57] Shah Z, Gulzar M, Hasan F and Shah AA. Degradation of polyester polyurethane by an indigenously developed consortium of *Pseudomonas and Bacillus* species isolated from soil. Polymer DegradStab. 2016;134: 349-356. Doi:10.1016/j.polymdegradstab.2016.11.003

[58] Barth M, Oeser T, Wei R, Then J, Schmidt J, Zimmermann W. Effect of hydrolysis products on the enzymatic degradation of polyethylene terephthalate nanoparticles by a polyester

hydrolase from *Thermobifidafusca*. BiochemEng J. 2015;93, 222-228. Doi:10.1016/j.bej.2014.10.012

[59] Li W-J, Jayakody LN, Franden MA, Wehrmann M, Daun T, Hauer B, Blank LM, beckhamGT, Klebensberger J, WierckxN. Laboratory evolution reveals the metabolic and regulatory basis of ethylene glycol metabolism by *Pseudomonas putida* KT2440. Environ Microbiol. 2019;21:3669-3682. Doi:10.1111/1462-2920.14703

[60] Gu J-D. Microbiological deterioration and degradation of synthetic polymeric materials: recent research advances. Int BiodeteriorBiodegrad. 2003;52 :69-91. Doi:10.1016/S0964-8305(02)00177-4

[61] Montazer Z, Habibi-Najafi MB, Levin DB. Microbial degradation of low-density polyethylene and synthesis of polyhydroxyalkanoate polymers. Can J Microbiol. 2019;65:1-11. Doi:10.1139/cjm-2018-0335

[62] Hou L, Majumder ELW. Potential for and distribution of enzymatic biodegradation of polystyrene by environmental microorganisms. Materials. 2021;14:503. Doi:10.3390/ma14030503

[63] Zhu B, Wang D, Wei N. Enzyme discovery and engineering for sustainable plastic recycling. Trends Biotechnology. 2022;40(1) :23-37. Doi:10.1016/j.tibtech.2021.02.008

[64] Alshehrei F.Biodegradation of synthetic and natural plastic by microorganisms. J Appl Environ Microbiol.2017;5(1):8-19. Doi:10.12691/jaem-5-1-2.

[65] Gambarini V, Pantos O, Kingsbury JM, Weaver L, Handley KM, Lear G. PlasticDB: a database of microorganisms and proteins linked to plastic biodegradation. Database, 2022; 1-12. Doi: Doi:10.1093/database/baac008

[66] Wei R and Zimmermann W. Microbial enzymes for the recycling of recalcitrant petroleum-based plastics: how far are we? MicrobBiotechnol. 2017; 10:1308-1322.Doi: 10.1111/1751-7915.12710

List of figures

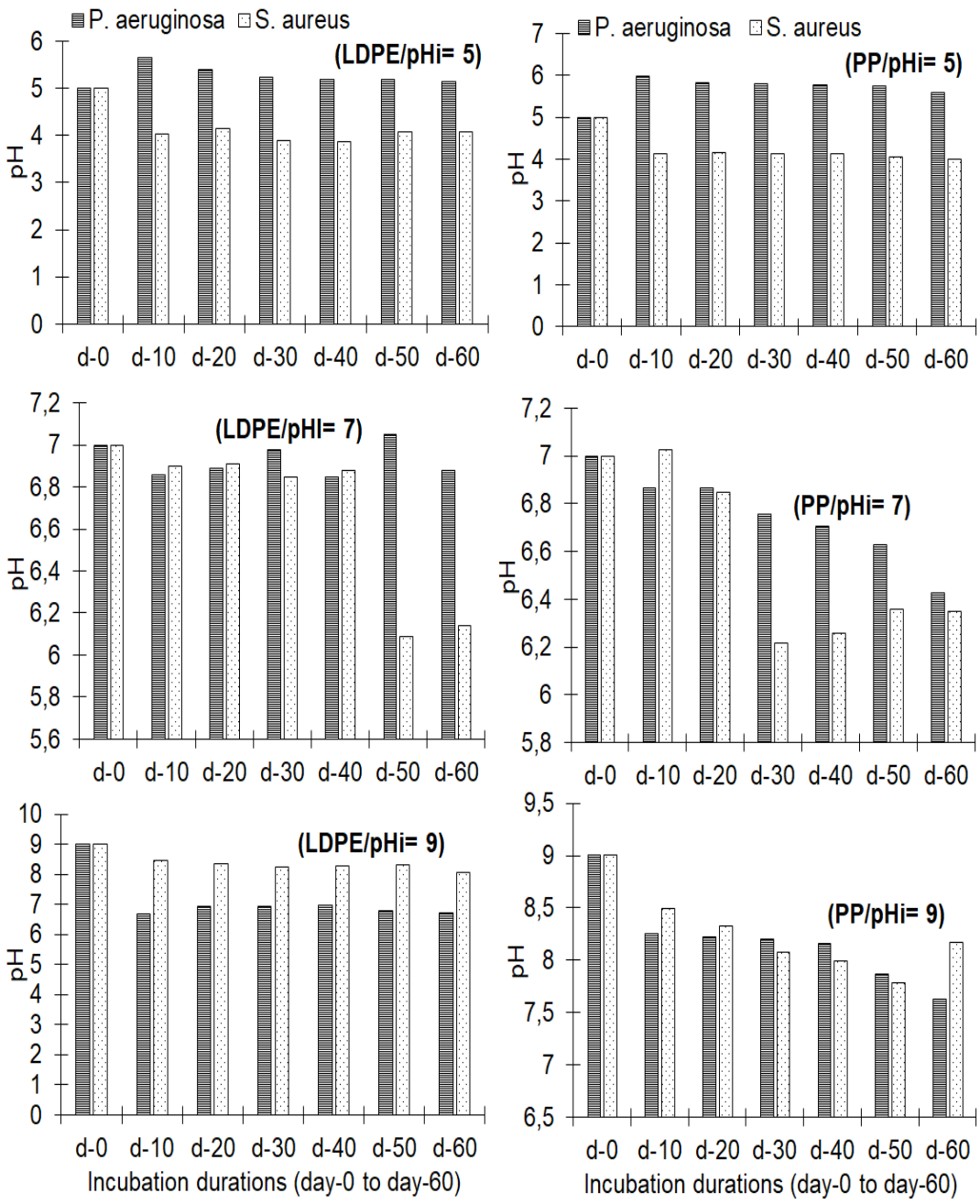

Fig. 1. Temporal variation of the pH of solutions containing the LDPE and PP fragments undereachpHi condition and in the presence of *P. aeruginosa* and *S. aureus*

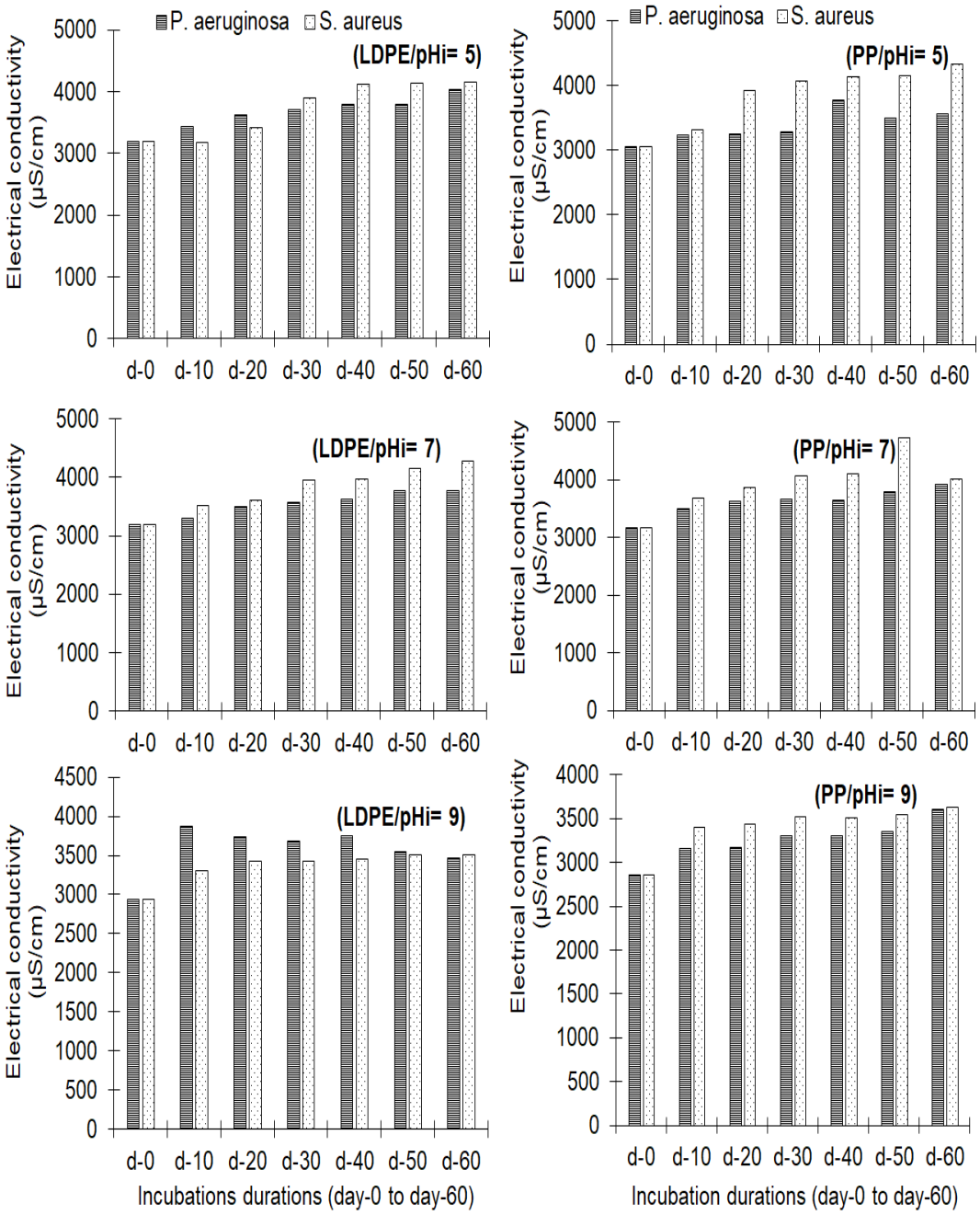

Fig. 2. Temporal variation of the electricalconductivity of solutions containing LDPE and PP fragments undereachpHi condition and in the presence of *P. aeruginosa* and *S. aureus*

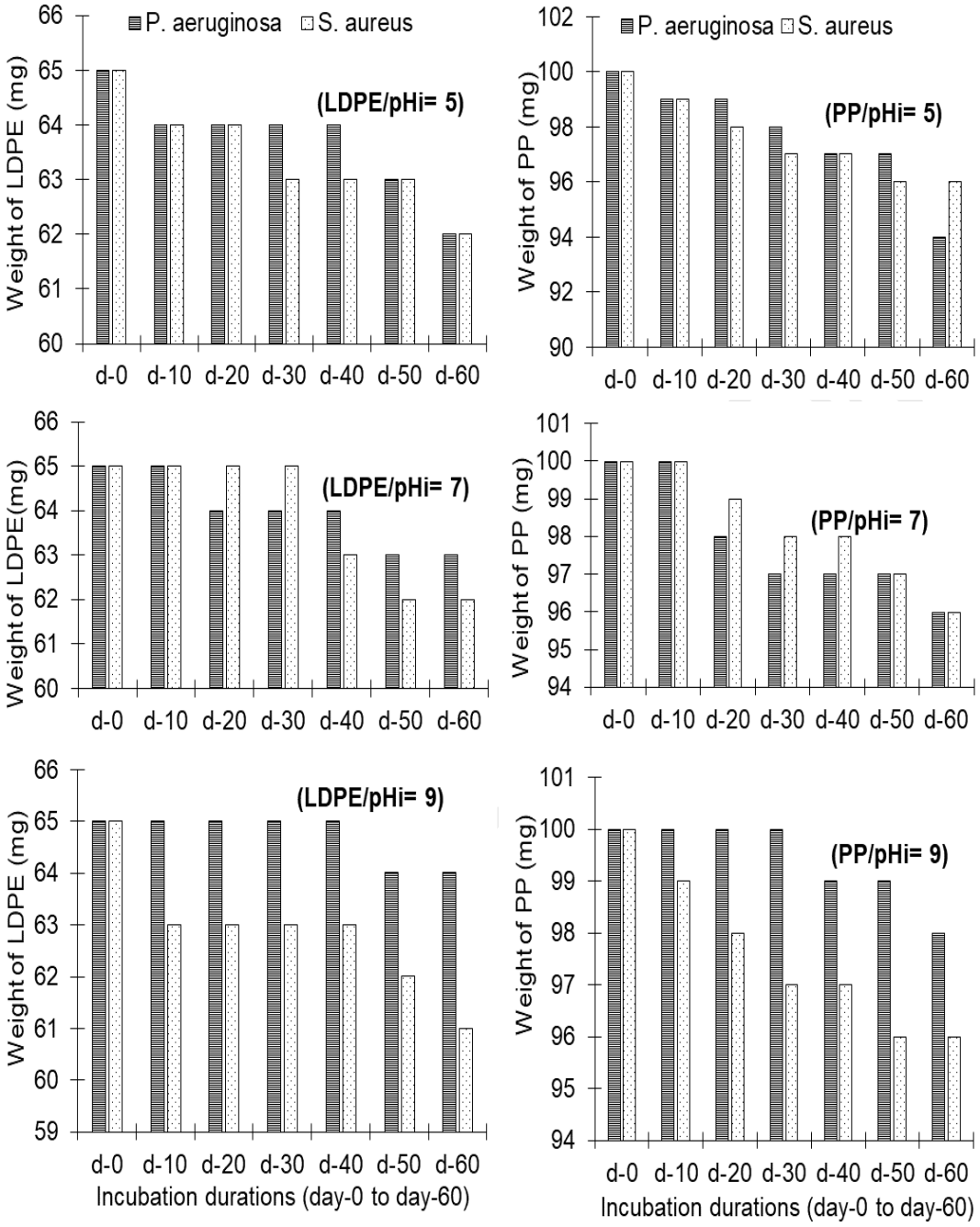

Fig. 3. Temporal variation of the weights of the LDPE and PP fragments undereachpHi condition of solutions and in the presence of *P. aeruginosa* and *S. aureus*

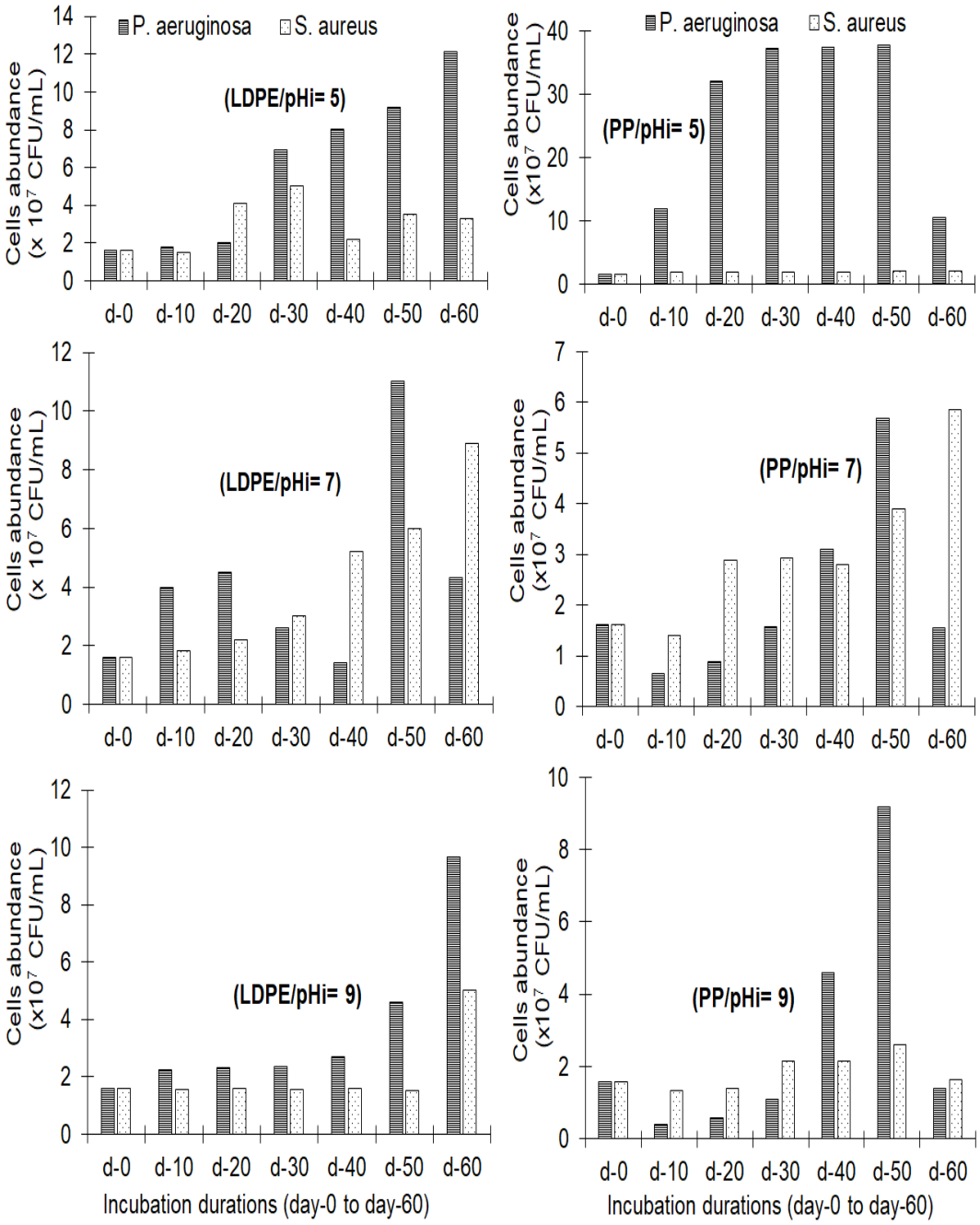

Fig. 4. Temporal variation of the of *P. aeruginosa* and *S. aureus*abundance in the presence of the LDPE and PP fragments and undereachpHi condition of solutions

List of Tables

Table 1.P values of the overallcomparison of the values of eachparameterrecordedduring the 7 incubation periods and for all 3 experimentalpHi conditions of the solutions

Type of polymer	Parameters considered and the bacterial species present in solution							
	Weight of fragments		pH		Electricalconductivity		Cellsabundance	
	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

Table 2. P values of the comparison for eachpolymerconsidered, between the values of eachparameterrecordedduring the 7 incubation periods in the presence of *P. aeruginosa* and thoserecorded in the presence of *S. aureus*, undereachpHi 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	Polymerweight	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***
	Cellsabundance	P=0.128	P= 0.455	**P=0.017***
PP	Polymerweight	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
	Cellsabundance	**P=0.000****	P=0.710	**P=0.017***

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

Table 3. Values of PWADR, ECAIR and CAAIR(and regression coefficient r^2) during incubations, in the presence of eachpolymer and eachbacterialspeciesconsidered, and undereachexperimentalpHi condition of solutions

Type of polymer and pHi value		Parameters considered and the bacterial species present in solution during					
		PWADR (mg/10 days)		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*