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Biodegradation of polyethylene by the bacterium *Pseudomonas aeruginosa* in acidic aquatic microcosm and effect of the environmental temperature


**Abstract**

The main problem about plastic packaging after their use is the fate of the plastic waste disposed of into the environment. This study was carried out in aquatic microcosm and aimed at assessing the impact of the temperature of an acidic environment on the polyethylene degradation by the bacterium *Pseudomonas aeruginosa*, as well as the electrical conductivity of the medium and the temporal abundance dynamics of cells involved. Sterilized pieces of polyethylene of 0.08 g in weight were immersed in sterile mineral solutions of pH 5 in glass flasks containing *P. aeruginosa* cells at concentration adjusted to 186.10^3 CFU/100 μL. The whole was incubated at 7 °C, 23 °C, 37 °C and 44 °C for 10 days, 20 days and 30 days. Analysis after incubations showed that electrical conductivity which was 3386 μS/cm at the initial moment increased with an increase in incubation period. Its highest value 5476 μS/cm was noted at 44 °C after 30 days. The pH of solutions decreased. Its lowest value 4.11 was noted at 7 °C after 10 and 20 days. The apparent degradation rates of polyethylene fragments varied from 8.10^{-5} g/10days (at 7 °C and 23 °C) to 2.10^{-4} g/10days (at 44 °C). The highest percentage of weight loss of the polyethylene was 6.25% registered after 30 days at 44 °C. The apparent changing rates in cell abundance varied with variation in incubation temperature. It was positive under 7 °C and 23 °C reflecting relative cell growth, and negative under 37 °C and 44 °C reflecting relative cell inhibition. The highest relative apparent cell growth rate was 1.831 CFU/10days recorded at 23 °C and the highest relative apparent cell inhibition rate was 7.831 CFU/10days recorded at 44 °C. In solutions, the pH, electrical conductivity and the weight of the fragments varied significantly from one incubation temperature to another and from one incubation period to another (P<0.05). Cell abundances varied only from one incubation temperature to another (*P* <0.05). The biodegradation process of polyethylene by *P. aeruginosa* under various environmental conditions and the impact of the products released on cells remains incompletely understood.

**Keywords:** Biodegradation, Polyethylene, *P. aeruginosa*, Acidic aquatic microcosm, Temperatures variation

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1. **Introduction**

The production of plastic packaging and their role in society has been known for several years. They are used as plastic bags, water and milk bottles, food packaging and toys (Sangale et al., 2012; Wilkes and Aristilde, 2017). The problem is with their fate in the environment after use. Changes in their properties in natural environment due to chemical, physical or biological reactions resulting in bond scissions and subsequent chemical transformations are categorized as polymer degradation. Depending on the nature of the causing agents, it has been classified as photo-oxidative degradation, thermal degradation, ozone-induced degradation, mechano-chemical degradation, catalytic degradation and biodegradation (Singh and Sharma, 2008).

According to Sivan (2011), biodegradable polymers are polymers whose chemical structure enables direct enzymatic degradation or mainly results in the microbial processes (Bonhomme et al., 2003). They are also called bioplastics. They are able to mineralize into Carbon dioxide, Methane, water, inorganic compounds (such as CO2, H2O, or CH4), or biomass through the enzymatic action of specific microorganisms such as bacteria and fungi under appropriate environmental conditions (Soroudi and Jakubowicz, 2013; Adhikari et al., 2016; Koch and Miha-
lyi, 2018; Ashok and Rejeesh, 2019). The process can either be aerobic or anaerobic. Aerobic microbes use Oxygen as an electron acceptor, and break down organic chemicals into smaller organic compounds, whereas anaerobic biodegradation is the breakdown of organic contaminants by microorganisms when Oxygen is not present (Gu, 2003; Priyanka and Archana, 2011). Polymers become brittle and crumble to small fragments and particles called microplastics (Sivan, 2011). They are plastic particles characterized by a diameter less than 5 mm and derived from macroplastics’ degradation. Some of them have been identified in food, drinking water, soil, and in the air (Folino et al., 2020).

Non-biodegradable plastics, usually known as synthetic plastics, are derived from petrochemicals. They are a continuous reoccurrence of small monomer units, thus have a very high molecular weight. On the other hand, biodegradable plastics are made from renewable resources which are completely biodegradable in their natural forms (Ghosh et al., 2013; Imre and Pukanszky, 2013; Alshehrei, 2017).

It is indicated that many bacteria species such as Pseudomonas fluorescens and P. aeruginosa show high ability to degrade polyethylene in aquatic environment under pH 7 and at 30–37 °C. The acidic pH seriously impacts negatively their activities compared to the alkaline condition (Hussein et al., 2015). A bacterial consortium (Pseudomonas putida, Pseudomonas fluorescens, Vibrio alginolyticus, Pseudomonas aeruginosa, Pseudomonas stutzeri, Flavobacterium species and Anaebana species) also degrade this plastic under slightly alkaline condition (pH 7.5) at 26 °C (Veethahavya et al., 2016). Other bacterial strains (Bacillus kruwicchiae, Bacillus pseudofirmus, Pseudomonas fluorescens, and Bacillus sp.) with unique capabilities to use low-density polyethylene as sole carbon source were isolated from a hyper alkaline spring (pH 11) (Dela Torre et al., 2018). This material can also be degraded in thermophilic condition by the appropriate bacterial strain as Brevibacillus borstelensis (Hdad et al., 2005). Others studies carried out under temperature ranged from 15 to 28 °C indicate that temperature is an important environmental factor that affects biodegradation rates, even though the apparent activation energy of the biodegradation reaction does not vary, showing persistency in the metabolic activities of the involved mesophilic microbial communities (Pischedda et al., 2019).

Despite this scattered information on microbial activity either under acidic or alkaline conditions, or under controlled temperature conditions regardless of pH, little data on the impact of varying the temperature of the medium on the biodegradation of this plastic and on the microbial biomass changes under acidic pH is available. Little is also known about the level of mineralization of the aquatic medium during the biodegradation process under controlled conditions. The present study aims at evaluating the evolution of pH, electrical conductivity and cell abundance related to the biodegradation of low density polyethylene under various controlled temperature in acidic aquatic environment.

2. Materials and methods

2.1. Making polyethylene fragments

Low density polyethylene of thickness 60 μm was used. It is a linear hydrocarbon polymer consisting of long chains of ethylene monomers (C2H4). It was cut into square fragments of 4 cm on each side. The weight of each fragment was adjusted to 0.08 g. The fragments were then immersed in a 70/30 (W/W%) water-ethanol mixture for sterilization according to Saadi (2008), 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 bacterium chosen for this study was Pseudomonas aeruginosa. It was chosen for its ability to use many carbon substrates as the sole source of carbon and energy in a simple mineral medium, its wide range of growth temperature, its ubiquitous character and its possibility of forming biofilms in environment (Hussein et al., 2015; Wilkes and Aristilde, 2017; Su et al., 2018).

The bacterium P. aeruginosa was isolated from surface water of Yaounde (Cameroon, Central Africa) using the membrane filtration method, on Cetrimide nalidixic acid agar culture medium (CN, Difo Laboratories, Detroit, MI, USA) contained in Petri dish. Incubation was done at 37 °C for 24 h. The typical P. aeruginosa colonies on CN agar medium were subsequently identified by using conventional biochemical tests (Holt et al., 2000; Gyung Yoon et al., 2012; Su et al., 2018).

2.3. Preparation of Pseudomonas aeruginosa culture

For the preparation of cell bacteria’s stocks, a colony forming unit (CFU) from CN agar medium was inoculated into 100 mL of nutrient broth (Oxford) for 24 h at 37 °C. After this period, cells were harvested by centrifugation at 8000 rev/min for 10 min at 10 °C and washed twice with NaCl (0.85%) solution. Each pellet was re-suspended in 50 mL of NaCl solution. After homogenization, 1 mL 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 growth of P. aeruginosa according to Saadi (2008) and Su et al. (2018), were thus dissolved in distilled water contained in Erlenmeyer flasks, in the required weight concentration. The pH was then adjusted to 5 using a pH-meter and 0.1 N HCl solution. 200 mL of the mineral solution was then distributed into each of the 48 glass flasks of 250 mL. 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 steriley introduced into each of the 48 vials. Then 2 mL of P. aeruginosa suspension compared to the Mc Farland solution previously prepared were introduced into 36 of the 48 flasks, then homogenized. The cells concentration was thus adjusted to 186.103 CFU/100 μL.

The 36 glass flasks containing the plastic fragments and bacterial cells were placed into 3 groups named A, B and C, each group having 12 glass flasks. The glass flasks of each group were then placed into 4 further subgroups. The glass flasks of group A were thus placed into subgroups A7, A23, A37 and A44. Those of group B were placed into subgroups B7, B23, B37 and B44, and those of group C into subgroups C7, C23, C37 and C44. Each subgroup consisted of 3 glass flasks. All the glass flasks of group A were incubated for 10 days, those of group B for 20 days, and those of group C incubated for 30 days. The triplets of the A7, B7 and C7 subgroups glass flasks were incubated at 7 °C. Those of subgroups A23, B23 and C23 were incubated at room temperature (23 ± 1°C). The triplets of flasks of subgroups A37, B37 and C37, and those of subgroups A44, B44 and C44, were incubated at 37 °C and 44 °C, respectively.

The other 12 glass flasks whose solutions contained the plastic fragments and devoid of bacterial cells served as controls. Each temperature and incubation duration thus had a control vial.
2.5. Biodegradability assessment of the polyethylene fragments

Several methods are used to assess the biodegradability of polymers fragments. These include, the formation of carbonyl group, the measurement of CO₂ production, estimation of bacterial abundance, quantitative estimation of the weight loss of the polymer fragments, changes in the chemical properties of the medium amongst others (Dela Torre et al., 2018; Rose et al., 2020; Min et al., 2020; Montazer et al., 2020). In this study, biodegradability was assessed using the last 3 criteria.

At the end of each incubation period and under each of the considered temperature, the solutions in flasks were vigorously stirred. This allows the homogenization of the solution and the detachment of the bacterial cells adhered to the polythene 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, then dried and its weight determined.

The abundance of cultivable bacterial cells in 100 μL of each solution was assessed using plate count method. This was done on standard agar culture medium in a Petri dishes. The colony forming units (CFU) were determined after incubation of 24–48 h at 37 °C.

2.6. Data analysis

The temporal variation of each studied parameter was illustrated using histogram. Relationships between all parameters were assessed using Spearman correlation tests. The comparisons of the measured parameters (Weight of the polythene, P. aeruginosa abundance, pH and electrical conductivity values of solutions) amongst the incubation durations and temperatures have been carried out using the H-test of Kruskal-Wallis. The weight loss (WL) of polyethylene was calculated using the following formula (Montazer et al., 2020):

\[ WL \% = \frac{(\text{Initial weight} – \text{Final weight})}{\text{Initial weight}} \times 100.\]

The apparent degradation rates of polyethylene fragments and the changing rates in cells’ abundance were assessed. The incubation period unit chosen was 10 days. The regression lines showing the temporal variations in the cells’ bacterial abundances and the temporal variations in the polymer weight at each incubation period (10 days, 20 days and 30 days) as a function of each experimental temperature were plotted. Each straight regression line equation (y = ax + b) was calculated using the method of least squares (Bailey, 1981; Tofallis, 2008; Popovic, 2019).

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 polyethylene fragments on one hand and to the changing rates in cell abundance on the other hand. 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 (Nola et al., 2012). Data analysis was performed using Statistical software package (SPSS 25.0).

3. Results

3.1. Temporal variation of the pH and electrical conductivity values in solutions

Fig. 1 (A and B) shows the temporal variations of the average values of electrical conductivity and pH of solutions containing both bacterial cells and polyethylene fragments subjected to the degradation process under various temperatures. The electrical conductivity of the solutions at the initial moment was 3386 μS/cm. After 10 days of incubation, it was 3482 μS/cm under 7 °C, 4293 μS/cm under 23 °C, 4363 μS/cm under 37 °C, and 5348 μS/cm under 44 °C. The same gradual increase with increasing incubation temperature was also recorded after 20 and 30 days (Fig. 1A). For the same incubation temperature, it was observed that the electrical conductivity increased with increasing incubation duration. Under 7 °C for example, it varied from 3386 μS/cm to 3482 μS/cm after 10 days of incubation, to 3519 μS/cm after 20 days and to 3606 μS/cm after 30 days of incubation. At room temperature (laboratory temperature), it is 4293 μS/cm after 10 days of incubation, 4367 μS/cm after 20 days of incubation, and 4387 μS/cm after 30 days of incubation. The same temporal and gradual increase of the electrical conductivity was noted at 37 °C and 44 °C. The highest value of the electrical conductivity was noted at 44 °C after 30 days of incubation (Fig. 1A).

For the pH, it decreased in all the solutions. At the end of each of the 3 incubation periods, the highest pH value was recorded under 37 °C. It was 4.94, 4.77 and 4.94 after 10, 20, and 30 days of incubation respectively (Fig. 1B). After 10 and 20 days, the lowest pH value was 4.11. This was noted when solutions were incubated under 7 °C. After 30 days, the lowest pH value registered was 4.47. It was noted when solutions were incubated under 44 °C. For all incubation periods, the highest pH values were recorded when the glass flasks containing cells and the polythene fragments were incubated under 37 °C (Fig. 1B).

3.2. Temporal variation in cells abundance

The averages of cells abundances fluctuated over time and with respect to the incubation temperature. At the initial moment, the cell abundance was 186.10³ CFU/100 μL. Under 7 °C of incubation, it changed to 181.10³ CFU/100 μL after 10 days, then to 261.10³ CFU/100 μL after 20 days, and to 184.10³ CFU/100 μL after 30 days of incubation (Fig. 2).

Under 23 °C, it changed to 266.10³ CFU/100 μL after 10 days, to 273.10³ CFU/100 μL after 20 days, and to 245.10³ CFU/100 μL after 30 days of incubation. The same patterns of temporal variations were observed when the glass flasks were incubated under 37 °C and 44 °C. Among all 3 incubation periods, the highest cell abundances were recorded under 23 °C, and the lowest under 44 °C (Fig. 2).

3.3. Temporal variation of weight of the polyethylene fragments

The average weight values of polyethylene fragments in the solutions varied with respect to the temperature and incubation duration (Fig. 3). The initial weight of polyethylene fragments which was 0.080 g decreased with increasing incubation period. Under 7 °C, the average weight of the fragments was 0.077 g after 10 and 20 days, and was 0.077 g after 30 days. The temporal decrease in fragment weight was recorded for each incubation temperature. Under each incubation temperature, the lowest weight of the fragments was recorded after 30 days of incubation. After 10 days, the lowest weight value was 0.077 g and this was recorded under 44 °C. After 20 and 30 days of incubation, it was 0.076 g and 0.075 g respectively, both recorded under 44 °C (Fig. 3).

3.4. Percentages of weight loss of the polyethylene fragments during incubations

Table 1 shows the percentage of weight loss of the polyethylene fragments under each incubation condition. It was observed that it varied with respect to the temperature and the incubation period. Under 7 °C, the percentages of weight loss was 1.25% after 10 days, 1.62% after 20 days and 3.38% after 30 days. Under 23 °C, it was 1.63%, 2.13% and 3.38% respectively after 10 days, 20 days and 30 days. The same profiles of the percentages of weight loss were recorded under 37 °C and 44 °C (Table 1). For each incubation, the highest percentage of weight loss was recorded under 44 °C incubation. It was 4.10% after 10 days, 5.00% after 20 days and 6.25% after 30 days (Table 1).
Fig. 1. Temporal variation of the electrical conductivity values (A) and the pH values (B) in solutions with respect to the fragments incubation temperatures in the presence of *P. aeruginosa* cells after each incubation period.

Fig. 2. Temporal variation of the mean values of cells abundance in solutions with respect to the polyethylene fragments incubation temperatures in the presence of *P. aeruginosa* cells after each incubation period.
Incubation temperatures & Incubation period & 10 days & 20 days & 30 days \\ 
\hline
7 °C & 1.25 & 1.62 & 3.34 \\ 
23 °C & 1.63 & 2.13 & 3.38 \\ 
37 °C & 2.88 & 4.13 & 5.37 \\ 
44 °C & 4.10 & 5.00 & 6.25 \\ 
\hline

3.5. Apparent degradation rates of polyethylene fragments and apparent changing rates in cells abundance

The apparent degradation rates of polyethylene fragments and the changing rates in cells abundance were assessed using the straight regression line method. The incubation period unit chosen was 10 days. The results are shown in Table 2. It appears that overall, the degradation rates of polyethylene fragments increased with increasing incubation temperature. The highest rate value was 2.10^{-5} g/10 days, and it was recorded under 44 °C. The lowest was 8.10^{-5} g/10 days and it was recorded under 7 °C and 23 °C.

The changing rates in cells abundance increased in general with the increasing of the incubation temperature. It was 0.740 CFU/10 days under 7 °C, 1.831 CFU/10 days under 23 °C. Under 37 °C and 44 °C, it was 1.784 CFU/10 days and 7.831 CFU/10 days respectively. However, under 7 °C and 23 °C, changing rates in cells abundance were positive whereas under 37 °C and 44 °C, they were negative (Table 2). This would mean that many bacterial cells died at the end of the incubation periods during the biodegradation process whereas many other cells grew during the process under 7 °C and 23 °C.

3.6. Correlations coefficients between the measured parameters

Spearman’s “r” correlation coefficients were evaluated between the parameters measured under each incubation temperature. Their values are presented in Table 3. It can be noted that under 7 °C, the profile of weights of the immersed polyethylene fragments and that of electrical conductivity of solutions are significantly opposite (P<0.01). The same observation is also made for the 2 parameters under 23 °C and 37 °C (P<0.01). However, the variation in weight of the polyethylene fragments and that of the pH values of the solutions are positively and very significantly correlated under 23 °C and 44 °C (P<0.05). Changes in the cells abundance under 44 °C and the variation of the weight of the immersed polyethylene are positively and significantly correlated (P<0.01); the same observations are made with the pH of solutions (P<0.01) (Table 3). The variation profiles of the electrical conductivity and pH are significantly opposite under 23 °C and 37 °C (P<0.01) (Table 3).

3.7. Comparison of the average values of all measured parameters amongst incubation durations and incubation temperatures

Comparisons of the average values of all parameters measured amongst incubation durations and incubation temperatures have been carried out using the Kruskal-Wallis H-test. The “P” values are presented in Table 4. It is noted that the pH, the electrical conductivity and the weight of the polyethylene fragments vary significantly from one incubation period to another (P<0.05). However, the variations in cells abundance with respect to the incubation durations are not very significant (P>0.05). All the parameters measured varied significantly from one incubation temperature to another (P<0.05) (Table 4).

4. Discussion

In solutions containing polyethylene fragments and *P. aeruginosa* cells, the electrical conductivity increased with increasing incubation period. This increase in electrical conductivity seem to be strongly influenced by the medium temperature. It has been indicated that under appropriate environmental conditions, specific microorganisms such as bacteria and fungi can mineralize polyethylene fragment into inorganic compounds through the enzymatic action. This would lead to the increase in the levels mineralization of solutions (Soroudi and Jakubowicz, 2013; Adhikari et al., 2016; Koch and Mihalyi, 2018; Ashok and Rejesh, 2019). In addition, polyethylene polymers contains some products that render them degradable. This include iron, cobalt, manganese, and calcium (Montazer et al., 2020).

The temperature of a medium is always as one of the key factors for the enzyme activity of the microorganisms present, regardless of
whether the enzymes are intracellular or extracellular. Since enzymes are biochemical catalysts, partially made up of protein, they are sensitive to heat in varying degrees. Raising temperatures of the environment generally multiplies the degree of enzyme activity. Once an optimum temperature has been reached, higher temperatures cause rapid degradation of the enzyme with concurrent and irreversible loss in activity (Zhang et al., 2018). For P. aeruginosa, optimal temperatures generally range from 30 °C to 44 °C (Hussein et al., 2015; Su et al., 2018). High temperatures generally have detrimental effects on the enzymes. However, there is broad variation in resistance and sensitivity to heat among the enzymes’ types (Engqvist, 2018).

Polyethylene plastic biodegradation involves the initial depolymerization and further degradation of the small molecules. It is however crucial to distinguish between enzymes that are involved in these two distinctive steps (Gan and Zhang, 2019). Various enzymes are often involved in the degradation of polyethylene. They include among others peroxidase, laccase, hydrolases, styrene monooxygenase, depolymerase, esterase, dehydrogenases, tannases, cutinases, lipases and carboxylesterases (Sangale et al., 2012; Wei et al., 2014; Wilkes and Aristidou, 2017; Danso et al., 2019; Mohan et al., 2020; Lee et al., 2020).

The variation in the activity of these enzymes in relation to the variation in temperatures and incubation periods would lead to the production of low molecular weight monomer and generation of carbon dioxide, methane, water molecule and ions in the medium; most of them such as carbon dioxide would be acidic (Arutchevli et al., 2008; Sangale et al., 2012, Roohi et al., 2017).

Yoshida et al. (2016) indicated that polyethylene digesting enzyme converts polyethylene to mono(2-hydroxyethyl) terephthalic acid (MHET), with minimal amounts of terephthalic acid (TPA) and bis(2-hydroxyethyl)-TPA as secondary products. Another enzyme, MHETase (MHET-digesting enzyme), further hydrolyzes MHET into the two monomers; TPA and ethylene glycol. Li et al. (2019) 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 would lead to the increase of the electrical conductivity of the medium as it has been noted in this study.

A gradual decrease in the weight of polyethylene fragments was observed with increase in incubation period and incubation temperature. This reflects a bacterial activity on these fragments.

Polyethylene degradation begins with the attachment of microbes to its surface followed by the production of some extracellular enzymes (Arutchevli et al., 2008). Under aerobic conditions, CO2, water and microbial biomass is the final degradation products whereas in case of anaerobic/ methanogenic condition CO2, water, methane and microbial biomass are the end products (Arutchevli et al., 2008; Sangale et al., 2012, Roohi et al., 2017).
2012). The conversion of the long chain polymer into CO2 and water is a complex process. Its degradation makes it fragile and sensitive to further oxidation by enzymes secreted by the microorganisms (Montazer et al., 2020).

The complete process of biodegradation has been divided into four stages (Mohanan et al., 2020): a)–biodeterioration, which is the formation of carbonyl-groups by the action of oxidative enzymes released by microorganisms or induced by external agents. Subsequent oxidation reduces the number of carbonyl-groups and generates carboxylic acids; 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) bio-mineralization, whereby small hydrocarbon fragments released by biofragmentation are taken-up and metabolized by bacteria, and d) mineralization, which is the transfer of hydrolys 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 bio-mineralization of small cleavage fragments by the microorganisms (Bonhomme et al., 2003; Montazer et al., 2019; Montazer et al., 2020). This would explain the increase in abundance of bacterial cells observed under 7 °C and 23 °C after 20 and 30 days of incubation, and under 23 °C after 10 days incubation.

The temperature 23 °C seems the most favorable for the cells. This has also been indicated by Saadí (2008). A decrease in cell abundance under 37 °C and 44 °C is observed at all incubation periods. Cellular activity or the recovery of the monomers from the medium for metabolism and growth would relatively be slowed down under general conditions of the medium both temperatures. Pischedda et al. (2019) noted during plastic biodegradation tests in the soil that an increase in the incubation temperature (from 15 °C to 28 °C) led to an increase in the concentration of CO2 released, the temporal variation of this CO2 concentration being in accordance with the Arrhenius equation. The mineralization rates varied from 0.00767 mg C/day/cm² at 15°C to 0.05421 mg C/day/cm² of plastic at 28 °C (Pischedda et al., 2019).

It has been indicated that P. aeruginosa shows high ability to degrade polyethylene in aquatic environment under pH 7 and at 30–35 °C, and the acidic pH has a serious negative impact on their activities compared to the alkaline condition ( Hussein et al., 2015; Veethahavya et al., 2016). In this study, it is noted that at acidic environment (pH 5), the biodegradation rates under 37 °C and 44 °C are lower than those registered under 7 °C and 23 °C. The effects of the acidity of the environment seem to be counterbalanced by lower temperatures.

The biodegradation is widely accepted as selective, and biodegradable plastics usually break down upon interaction with UV, water, temperature, oxygen content, time, nutrient availability, en- zymes, microorganisms’ presence and gradual changes in pH (Alshehrei, 2017; Folino et al., 2020). It also depends on the physical and chemical properties of biopolymers and the thickness of the biodegradable material (Rujnic-Sokele and Pilipovic, 2017; Wahyuningtyas and Suryanto, 2017; Meerbeek et al., 2020). These are linked to a)-the availability of functional groups that increase hydrophobicity (hydrophilic degradation is faster than hydrophobic), b)-the molecular weight and density of the polymer (lower degrades faster than higher), c)-the amount of crystalline and amorphous regions (amorphous degrades faster than crystalline), d)-the structural complexity such as linearity or the presence of branching in the polymer, e)-the presence of easily breakable bonds such as ester or amide bonds, f)-the molecular composition, g))the nature and physical form of the polymer (e.g., films, pellets, powder or fibers), h)-hardness (soft polymers degrade faster than hard ones), i)-transition temperature and melting temperature of the polymers (Mohan and Srivastava, 2010; Batori et al., 2018; Folino et al., 2020).

It is indicated that plastic polymers can be broken down to varying degrees both physically and biologically with minimal generation of compounds amenable to metabolism inside the cells. Intermediate products produced from the first steps of biodegradation can sometime interfere with future steps needed for uptake and subsequent intracellular metabolism (Kolvenbach et al., 2014; Barth et al., 2016; Wilkes and Aristide, 2017). The result of this interference would vary depending on environmental conditions, such as temperature and pH. Under some environmental conditions, some enzymes found in cells can also inhibit degradation. This is the case of the bacterium Flavobacterium sp (Barth et al., 2016; Wilkes and Aristide, 2017). This would explain the relative variations of biodegradation rates of the immersed polyethylene fragments as well as the rates of changes in cell abundance.

5. Conclusion

Polyethylene plastics play an important role to humans in society. But they are very harmful when they are released as waste into the environment. Their degradation can be carried out biologically or non- biologically. Their biodegradation by the bacterium P. aeruginosa, for example, is strongly influenced by environmental conditions. The effects of an acidic pH of the environment, known to slow down the biodegra- dation of polyethylene plastic by this bacterium seem to be counterbal- anced by low temperatures in the environment. The degradation of this polymer by this bacterium leads to its growth in the medium. However, at temperatures conventionally optimal for its growth rate, its multipli- cation seems to be slowed down, probably as a result of the chemical composition of the medium which has become metabolically less favor- able. Potential inhibitory effects of plastic degradation by-products on P. aeruginosa under various conditions remain to be fully understood.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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