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Mineralization of ^{13}C-labeled polyethylene by marine *Bacillus velezensis* MT9

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ABSTRACT

In the marine environment, plastic debris breaks down into smaller entities known as microplastics. Polyethylene (PE) is the main source of microplastics. In a previous study, we showed that marine *Bacillus* MT9 and *Vreelandella* strains (MT1, MT11) were able to degrade untreated PE based on SEM, gravimetric weight loss and FTIR analysis. In this study, we performed stable isotope tracing assays, measuring the production of $^{13}CO_2$ from ^{13}C-PE powder (untreated or UV-treated) incubated with *B. velezensis* MT9, *V. titanicae* MT11 or *V. venusta* MT1 isolates under aerobic conditions in the presence and absence of yeast extract as co-nutrient source. Only *B. velezensis* MT9 exhibited mineralization activities towards untreated and UV-treated ^{13}C-PE with the highest ^{13}C-mass loss of 0.199% recorded for UV-treated ^{13}C-PE with yeast extract, after 14 days incubation. In this study, we demonstrated the mineralization of ^{13}C-PE by *B. velezensis* MT9. Furthermore, we also confirmed that photodegradation of plastic is a key process to enhance the biodegradation of PE. These findings suggest that *Bacillus* sp. could potentially degrade PE plastic waste in marine environment, as they can slowly mineralize PE even when other nutrients are present.

1. Introduction and study objectives

Plastics play a crucial role in the global economy with a current annual production rate of >400 million tons per year (Geyer et al., 2017; Hofmann et al., 2023; Plastics Europe, 2024). However, inadequate have resulted in the accumulation of large amounts of plastic in the environment Specifically, approximately 5–13 Mt of plastic enter the ocean annually (Geyer et al., 2017; Auta et al., 2017) and this number is projected to increase 2.6-fold by 2040 (Lau et al., 2020).

The fate of plastic in the ocean remains poorly understood but microbial biodegradation might be one of the processes contributing to mitigate environmental risks related to plastic pollution in marine environments.

Under this scenario, recent microbiological research has aimed to investigate whether and to what extent microorganisms can degrade plastics (Putar et al., 2025; Tian et al., 2017). Particular attention has been dedicated to PE, which has been identified to be the most abundant polymeric material among the microplastics recovered from the marine environment (Suaria et al., 2016). PE biodegradation is often the result of combined abiotic and biotic processes (Pandey et al., 2023; Zhang et al., 2025). Weathering processes and especially photodegradation are currently recognized as the primary forces driving the degradation of hydrophobic plastics like PE. This leads to alterations in the chemical, physical, and mechanical properties of the plastics. Subsequently, smaller degradation products, such as long and short-chain alkanes, compounds containing carbonyl groups, oligomers, micro and nano plastics, may undergo biomineralization by microbes (Vaksmaa et al., 2023). Microorganisms have been widely reported for their role in plastic degradation (Gao et al., 2022; Giacomucci et al., 2020; Khandare et al., 2022, 2021; Paço et al., 2017; Ramanayaka et al., 2024; Restrepo-Flórez et al., 2014; Salinas et al., 2025; Syranidou et al., 2019, 2017; Tian et al., 2017; Wayman and Niemann, 2021; Khampratueng and Anal, 2026; X. Chen et al., 2025; Dhanraj et al., 2025; Trad et al., 2025).

However, most of the available reports on PE biodegradation deal with terrestrial habitat while only few studies reported PE biodegradation in marine environments (Bajo et al., 2024; J. Chen et al., 2025; Gao and Sun, 2021; Raddadi and Fava, 2019; Sardar, 2025; Sun et al., 2025; Wayman and Niemann, 2021; Zhang et al., 2025). Among fungi, few marine species belonging to the genera *Zalerion* (Paço et al., 2017), *Alternaria* (Gao and Sun, 2021), *Rhodotorula* (Vaksmaa et al., 2023) and

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Parengyodontium (Vaksmaa et al., 2024), have so far been shown to degrade PE. With regard to the marine bacteria, *Bacillus* sp. and *Vreelandella* sp (previously *Halomonas* sp.) strains were reported as LDPE degraders (X. Chen et al., 2025; Dhanraj et al., 2025; Khandare et al., 2021; Kumari et al., 2019; Oren and Göker, 2024; Syranidou et al., 2019). Harshvardhan et al. (2013) isolated two LDPE degrading strains, *Bacillus pumilus* and *Bacillus subtilis*, from pelagic waters of the Arabian Sea that were able to exhibit a mass loss of autoclaved LDPE commercial bags of up to 1.75% after 30-days incubation. Khandare et al. (2021) reported a marine *Halomonas* sp. demonstrating a maximum weight loss of unpretreated commercial LDPE film of 0.78 and 1.72% after 30- and 90-days aerobic incubation, respectively. An artificial marine bacterial consortium consisting of *Exiguobacterium* sp., *Halomonas* sp., and *Ochrobactrum* sp. degrades LDPE film with approximately 47 % reduction in molecular weight after two weeks incubation (Gao and Sun, 2021). Joshi et al. (2022) described marine *Bacillus spp., Paenibacillus spp., Shewanella, Rheinheimera, Oceanimonas and Vibrio* spp as able to degrade LDPE. Recently, Rong et al. (2024a) isolated from seawater samples a *Rhodococcus qingshengii* strain able to degrade LDPE (Rong et al., 2024a). Additionally, Rong et al. (2024b) were enriched and isolated two strains, from coastal plastic debris two strains, *Nitratireductor* sp. Z-1 and *Gordonia* sp. Z-2 which were able to degrade LDPE film, within 30 days incubation (Rong et al., 2024b). *Pseudalkalibacillus* able to degrade PE was isolated by Meng et al. (2024) from marine environment. Regarding *Bacillus velezensis*, few isolates obtained from marine environment have been reported as able to degrade polyurethane (Gui et al., 2023) or LLDPE (Bajo et al., 2024). The ability of *B. velezensis* to degrade untreated commercial polyethylene wrap has been reported for a landfill isolate (Liu et al., 2022). The biodegradative capacity of the strain was highly improved when PE was subjected to Cobalt catalysed carbonyl functionalization (Tang et al., 2024). Hence reports dealing with PE biodegradation by marine *B. velezensis* are very scarce.

Moreover, the commonly employed approaches to measure microbial plastic degradation and to compare results across studies are rooted in methodological difficulties (Montazer et al., 2020). For example, gravimetric weight loss measurement or Fourier-transform infrared spectroscopy for polymer degradation assessment face limitations. Indeed, gravimetric weight loss results can be affected by high inaccuracy due to the low weight losses often observed, the presence of additives, the molecular weight distribution, the crystallinity and/or fragmentation/disintegration of plastics particularly weathered. On the other hand, FTIR spectroscopy, used for the detection of the presence of functional groups on the plastic surface does not allow the determination of the extent/rate of biodegradation. Furthermore, imaging techniques like scanning electron microscopy or atomic force microscopy cannot precisely discriminate abiotic degradation from biotic one, especially when the degradation extent is low, because of their lack in sensitivity implying time-intensive procedures (Ghatge et al., 2020; Montazer et al., 2020; Rong et al., 2024a; Tian et al., 2017). Overall, these approaches are inadequate for determining microbial degradation kinetics. In addition, they do not allow direct tracing the fate of carbon atoms from the polymer to degradation products or microbial biomass. Therefore, methods that enable measuring low biodegradation rates need to be (further) explored (Ghatge et al., 2020).

In the past decades, ^{14}C labelled plastics were used to test biodegradation/mineralization of PE and polystyrene by measuring $^{14}CO_2$ generation and release of dissolved ^{14}C-compounds (Guillet et al., 1974). Due to lack of availability, ^{14}C labelled plastics have not been tested during last two decades. Only recent studies assessed unambiguously the PE mineralization by *Rhodococcus ruber* strain C208 (Goudriaan et al., 2023), a marine yeast *Rhodotorula mucilaginosa* (Vaksmaa et al., 2023) and a marine mould *Parengyodontium album* (Vaksmaa et al., 2024) using stable isotope tracing assay with ^{13}C-labelled polyethylene. The results of these studies showed degradation rates of up to 1.2% yr^{-1}; 3.8% yr^{-1} and 0.044% day^{-1}, respectively. The attack of PE by *Bacillus* sp. and *Vreelandella* sp. (the new genus where some *Halomonas* species have been reclassified) was demonstrated by observing changes in the chemical (Fourier Transform InfraRed spectrometry (FTIR) and structural properties (scanning electron microscopy (SEM) and atomic force microscopy (ATM)) of PE recovered at the end of the incubation period (Bajo et al., 2024; Bitalac et al., 2023; Chamas et al., 2020; Gao and Sun, 2021; Ghatge et al., 2020; Khandare et al., 2021; Kumari et al., 2019; Syranidou et al., 2019). Nevertheless, to our knowledge, there are no reports that can prove their ability to mineralize PE.

Our study aimed to demonstrate biomineralization of untreated and/or UV-treated ^{13}C-PE incubated in mineral medium with and without the addition of a co-nutrient source (yeast extract) by marine *Bacillus* sp. and *Vreelandella* sp.

2. Materials and methods

2.1. Bacterial strains

The bacterial strains were isolated from marine environment as outlined in our previous work (Bajo et al., 2024). The isolates were identified by *gyrB* gene sequencing as *Bacillus velezensis* isolate MT9 (PQ096961), *Vreelandella venusta* isolate MT1 (PQ096959) and *Vreelandella titanicae* isolate MT11 (PQ096958).

2.2. ^{13}C-polyethylene plastic

The plastic powder used was PE labelled with the stable isotope ^{13}C; (poly(ethylene-$^{13}C_2$), 99.2 atom-%, Sigma Aldrich, Batch #: MBBD1989; with a granular diameter of less than 1 mm and a melting point of 121 °C. The ^{13}C-PE powder was analysed by FTIR to confirm both the plastic identity and the isotope labelling. This analysis was conducted using an Agilent's Cary 630 spectrometer operating in diamond Attenuated Total Reflectance (ATR) mode, and the spectra were recorded over the wavelength range of 650–4000 cm^{-1}.

2.3. UV-pretreatment of ^{13}C-polyethylene

PE floats in marine environment due to its lower density than seawater, leading to UV light exposure that causes photochemical alterations. To simulate this natural process, 1 mg of ^{13}C-PE powder was subjected to UV light using a Blue Wave 200 Version 3.0 upratech HTC 400-241 lamp. This lamp emits a UV spectrum within the 300–450 nm range (UV-A). The irradiance levels were maintained at ~200 mW cm^{-2} (2000 W m^{-2}), and the plastic samples were exposed to UV light for approximately 15 h. This UV dose is equal to approximately 50 days of radiation at the sea surface in subtropical oceans or ~125 days in temperate marine regions, where UV irradiance levels are around 25 W m^{-2} and 10 W m^{-2}, respectively (Li et al., 2015). The UV treatment of the plastic was carried out in a 50 mL Pyrex beaker containing 50 mL of saltwater (30 g/L NaCl in distilled water). The setup included controlled stirring at 60 rpm using inert glass stirring bars, while the temperature was kept stable at approximately 24 °C up to the end of the experiment. At the conclusion of the UV exposure, we measured a minimal temperature increase in the liquid up to 5 °C and this is consistent with the minimal water evaporation during the experiment (15-h incubation period). FTIR spectra were recorded for the UV treated ^{13}C-PE to assess photochemical alteration on the polymer surface.

2.4. ^{13}C-biodegradation assays

The biodegradation experiments were set up in 125 mL borosilicate serum bottles with 20 mL Brunner Medium (BM) supplemented with 1 mg of untreated or UV-treated ^{13}C-PE previously sterilized by autoclaving. Then serum bottles were sealed with rubber butyl stoppers (20 mm) and aluminium crimp under aerobic conditions. The BM

composition was (g/L): KH_2PO_4, 1.52; Na_2HPO_4, 2.44; $(NH_4)_2SO_4$, 0.5; $MgSO_4{\cdot}7H_2O$, 0.2; $CaCl_2$, 0.05; NaCl, 30; $NaHCO_3$, 0.84. A 10 mL/L trace element solution with the following composition of (g/L) $FeSO_4{\cdot}7H_2O$, 0.2; EDTA, 0.5; $ZnSO_4{\cdot}7H_20$, 0.01; $MnCl_2{\cdot}4H_2O$, 0.003; H_3BO_3, 0.03; $CoCl_2{\cdot}6H_2O$, 0.02; $CuCl_2{\cdot}2H_2O$, 0.001; $NiCl_2{\cdot}6H_2O$, 0.002; $Na_2MoO_4{\cdot}2H_2O$, 0.003 was supplemented. Moreover, 2.5 mL/L of a vitamin solution was added, the solution contained (g/L) *p*-Aminobenzoate, 0.01; biotin, 0.002; nicotinic acid, 0.02; thiamine-HCl·$2H_2O$, 0.01; Ca-pantothenate, 0.005, vitamin B_{12}, 0.02. Two separate assays were performed. The first approach involved the incubation of the three bacteria *i.e.*, *B. velezensis* isolate MT9, *V. venusta* isolate MT1 and *V. titanicae* isolate MT11 in BM supplemented with untreated ^{13}C-PE and 0.5 g/L of yeast extract. In addition, a set of biotic controls was established by incubating the strains in BM without ^{13}C-PE and abiotic controls by incubating the labelled plastic powder in the same media without inoculating the strains. Subsequently, based on the mineralization results, the most promising strain was selected for further investigation with UV-treated ^{13}C-PE under two different scenarios: I) BM + ^{13}C-PE(+UV) + yeast extract (0.5 g/L) and II) BM + ^{13}C-PE(+UV) without yeast extract with the respective biotic and abiotic controls. The serum bottles were inoculated with a bacterial pre-inoculum prepared by transferring single colonies from an overnight culture growth on nutrient agar plates at 30° to 100 mL flasks containing 20 mL of Tryptic Soy Broth (TSB) amended with 30 g/L NaCl medium. After reaching mid-log phase during overnight incubation at 30 °C (150 rpm), the pre-inoculum biomass was recovered by centrifugation (6000 rpm, 10 min, 15 °C), washed three times with the corresponding BM formulations, *i.e.*, with or without yeast extract, and inoculated at a final concentration of approximately 10^7 CFU/mL. Biodegradation experiments were carried out at 30 °C and 150 rpm for up to 140 and 60 days for untreated and UV-treated plastic, respectively.

2.5. Evaluation of bacterial growth and endospores formation

Bacterial growth was monitored at various time intervals using the drop plate method as reported by (Bajo et al., 2024). The growth curves were represented by plotting the $\log_{10}$ CFU/mL as a function of time (days). Sporulation was checked by microscopy observation of culture samples stained using the Schaeffer-Fulton method as described by (Hussey, 2013). Under these conditions, spores are stained green and vegetative cells are stained red.

2.6. ^{13}C-polyethylene mineralization measurement

In this study, the PE used is labelled with ^{13}C stable isotope and as direct consequence, the ability of the isolate to use the plastic substrate as energy source is reflected by an increment of the $^{13}CO_2$ as terminal oxidation product in the carbonate pool (Goudriaan et al., 2023; Maier et al., 2026; Vaksmaa et al., 2023). The accumulation of $^{13}CO_2$ leads to variations in the natural isotopic abundance, where ^{12}C is more abundant (98.9 %) than ^{13}C (1.1 %). Therefore, to precisely measure variation in the isotopic ratio $^{13}C/^{12}C$ due to microbial activity we measured the carbon isotopic identity in the dissolved inorganic carbon (DIC) (Vaksmaa et al., 2023, 2024; Goudriaan et al., 2023; Maier et al., 2026). For this purpose, a Picarro G2131i gas analyzer (California, USA), based on wavelength-scanned cavity ring-down spectroscopy (WS-CRDS), was used. This instrument offers an excellent detection sensitivity (1 ‰) by recirculating many times the light through the sample by improving the light-sample interaction. Specifically, at different incubation times under different experimental conditions a volume of 0.5 mL of culture broth was collected and added to an Exetainer® 12 mL vial sealed with a cap (Labco, United Kingdom). Then, 2 mL of 10 % H_3PO_4 and 2 mL of deionized water were injected separately into the vial to release the $^{12}CO_2$ -$^{13}CO_2$ mixture in solution by using an AutoMate Prep Device (set to 2 s each). The released CO_2 was then automatically transferred to the WS-CRDS analyzer, and the C-isotope ratio detected.

2.6.1. Quantification of polyethylene mineralization

The δ^{13}C values measured by the gas analyser were calculated based on internal calibration using standard gas and then expressed in parts per thousand (‰) by delta notations relative to the Vienna Pee Dee Belemnite (VPDB) standard (0.0111796), as below:

$$\boldsymbol{\delta^{13}C} = \left(\frac{\mathbf{R}_{sample}}{\mathbf{R}_{standard}} - \mathbf{1}\right) \times \mathbf{1000} \quad (1)$$

where R_{sample} and $R_{standard}$ represent the isotope carbon ratios in the sample and standard, respectively:

$$^{13}\boldsymbol{R} = \frac{^{13}\boldsymbol{C}}{^{12}\boldsymbol{C}} \quad (2)$$

The headspace (HS) concentration was determined based on the DIC CO_2 in the liquid phase considering their equilibrium as described by Zeebe and Wolf-Gladrow (2001). Subsequently, both CO_2 concentrations in the HS and DIC were transformed into the total CO_2 amount per incubation serum bottle (ΣCO_2). The excess in ^{13}C in the total carbon pool (CO_2_DIC + CO_2-HS+10 mM $NaH^{12}CO_3$) was calculated from the change in δ^{13}C, which corresponds to changes in the fractional abundance (^{13}F) of ^{13}C (Hayes, 2004).

(3)

To determine microbially mediated mineralization rates, the ^{13}C excess ($^{13}C_{ex}$) was quantified assuming that an increase in ^{13}F in the ΣCO_2 pool is caused by an excess amount of ^{13}C originated from the added target labelled plastic:

$$^{13}\boldsymbol{C_{ex}} = \left[\left(^{13}\boldsymbol{F}\right)_{\mathbf{end}}\right] - \left[\left(^{13}\boldsymbol{F}\right)_{\boldsymbol{to}}\right] \times \left(\sum \boldsymbol{CO_2}\right) \quad (4)$$

Where $(^{13}F)_{end}$ and $(^{13}F)_{to}$ represent the fractional abundance of ^{13}C in the ΣCO_2 at the beginning (t_0) and at the end (t_n) of the experiment and (ΣCO_2) the total CO_2. Moreover, we corrected our calculations considering the possible spontaneous accumulation of ^{13}C in the abiotic conditions especially due to ongoing radical chain reaction leading to polymer oxidation and CO_2 production even after UV exposure was interrupted. The absolute ^{13}C production ($^{13}C_{ex}$ (net)) was expressed as:

$$^{13}\boldsymbol{C_{ex}}(\boldsymbol{net}) = {}^{13}\boldsymbol{C_{ex}}(\boldsymbol{biotic}) - {}^{13}\boldsymbol{C_{ex}}(\boldsymbol{abiotic}) \quad (5)$$

The changes over time of ^{13}C net excess expressed in μmol was then translated into ^{13}C net excess expressed in μg based on the molecular weight of PE-monomer -($^{13}C_2H_4$)- and the $^{13}CO_2$ accumulation per incubation that follows this stoichiometric equation:

$$-\left(^{13}\boldsymbol{C_2H_4}\right) - + \mathbf{3}\boldsymbol{O_2} \rightarrow \mathbf{2}\ ^{13}\boldsymbol{CO_2} + \mathbf{2}\ \boldsymbol{H_2O} \quad (6)$$

Then, the percentage of degradation (%) at different incubation times was calculated based on the initially added ^{13}C-PE (1 mg) (Goudriaan et al., 2023; Vaksmaa et al., 2023).

2.7. Statistical analyses

Significance analysis was performed using GraphPad Prism version 8.0.0 for Windows (GraphPad Software, San Diego, CA, https://www.graphpad.com) and a statistical probability $p < 0.05$ was considered significant using one-way ANOVA followed by Tukey's multiple comparisons test. All experiments were performed in triplicate (UV- treated ^{13}C-PE) or quadruplicate (untreated ^{13}C-PE). Data are expressed as mean ± SD.

3. Results and discussion

In this work, the PE biomineralization potential of 3 marine bacterial pure isolates was carried out through a stable isotope assay with ^{13}C-labelled PE. In our previous work, we demonstrated the LLDPE biodegradation potential of *B. velezensis* MT9, *V. titanicae* MT11 and *V. venusta* MT1 isolates via gravimetric film weight loss (%) evaluation, SEM observation and/or ATR-FTIR analysis on the incubated films. However, these methods do not trace carbon from the polymer into terminal degradation products, which is crucial for providing unequivocal evidence of plastic biomineralization (Montazer et al., 2020). Therefore, stable isotope assays were carried out on the mentioned cultures to assess their PE mineralization potential. The PE mineralization assays were carried out with both untreated ^{13}C-PE and on UV-treated ^{13}C-PE, to simulate the natural plastic-photooxidation that occurs in actual site marine environments.

3.1. Chemical characterization of untreated and UV-treated ^{13}C-polyethylene

The ^{13}C-PE powder identity analysis was carried out using ATR-FTIR. The spectrum of the labelled plastic was recorded and then compared with a reference ^{12}C-PE film to assess the distinctive PE peaks. The ^{13}C-PE exhibits characteristic PE spectra having peaks that fall in the regions of 2919 and 2850 cm-1 (-CH), 1460 cm-1 and 1470 cm-1 (-CH), and approximately 720–730 cm−1 (-CH), respectively (Gui et al., 2023; Meng et al., 2024; Syranidou et al., 2019). The position of peaks in a FTIR spectrum depends on the atomic mass of the isotopes involved. Given the higher atomic mass of carbon-13 with respect to carbon-12, the peaks corresponding to ^{13}C-PE (99% ^{13}C) showed an isotopic shift of -^{13}CH peaks at a slightly lower frequency with respect to -^{12}CH (Fig. 1S.).

As aforementioned, the ^{13}C-PE was also exposed to UV light under two different conditions i.e. for 15 h in salt water or for 2.5 h (e.g., for an exposure time six-time shorter than the 15 h duration of the treatment in salt water) under dry conditions. Afterwards, FTIR analyses were performed for evaluating changes (i.e. the appearance of functional groups and their intensity) on the surface of the polymer. The spectra obtained from ^{13}C-PE dry + UV 2.5h and ^{13}C-PE salt water + UV 15h are displayed in Fig. 1. After 15h exposure, the PE showed a FTIR marked absorption band around 1700 cm^{-1} that falls in the region of C=O group (Kane and Clare, 2019; Paço et al., 2017). In addition, several low-intensity bands due to the stretching vibrations of C-O groups of the alcohol group (-OH) were detected in the range 1275-1025 cm^{-1} (Paço et al., 2017; Vaksmaa et al., 2023). On the other hand, in the dry conditions, only the C=O band showed similar intensity in saltwater and dry condition while a more pronounced C-O stretching band and a new peak at 3340 cm^{-1}, within the hydroxyl group spectrum range (3200-3600 cm^{-1}) (Gui et al., 2023; Meng et al., 2024; Syranidou et al., 2019), have been observed. These results indicate that the spectra of the ^{13}C-PE salt

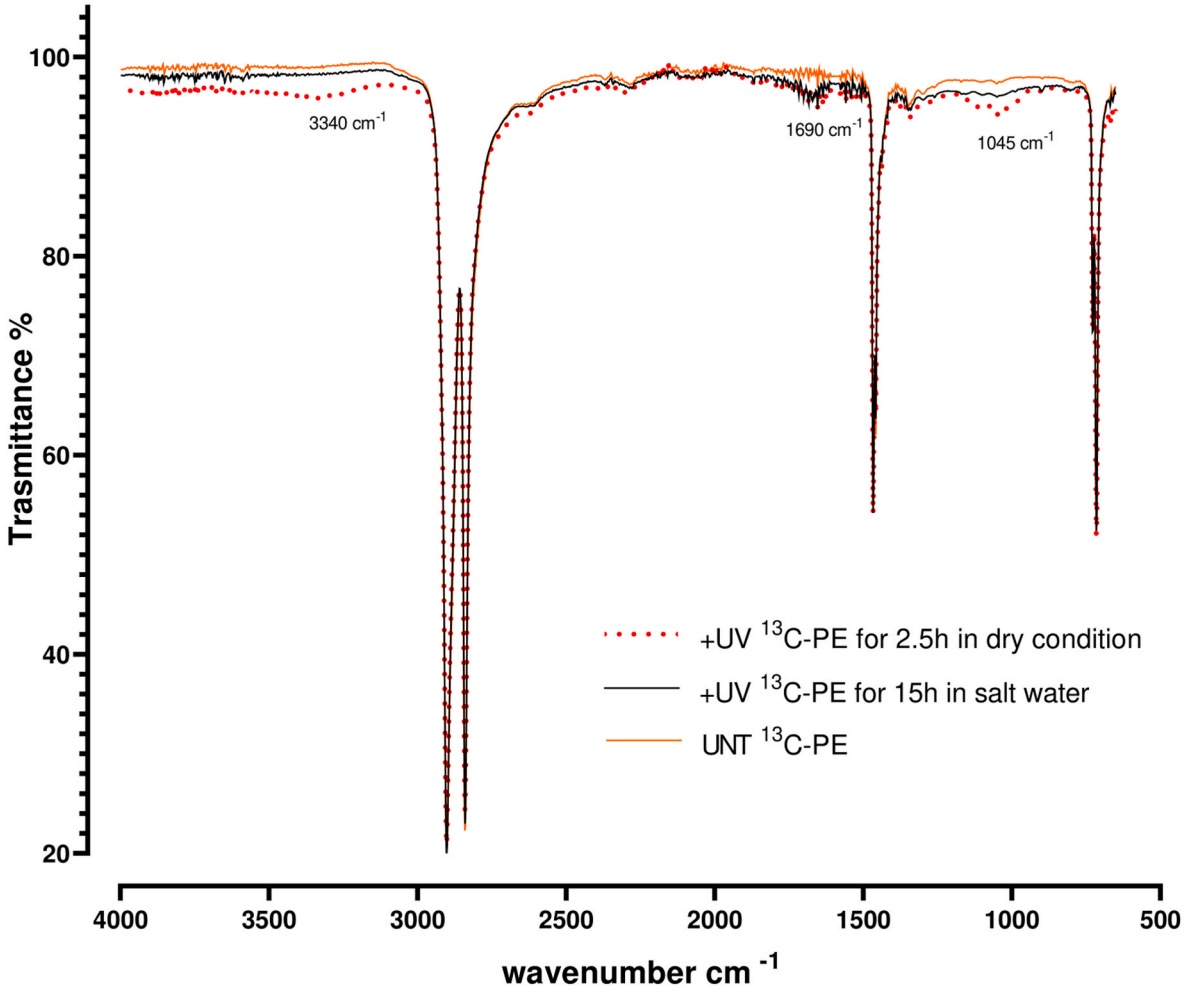

Fig. 1. FTIR spectra of ^{13}C-PE treated under dry conditions with UV exposure for 2.5 h (dashed red line), of ^{13}C-PE exposed to saltwater with UV treatment for 15 h (black line) and of untreated ^{13}C-PE (orange line). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

water + UV 15h exhibit lower chemical alterations than the ^{13}C-PE dry + UV 2.5h even if the exposure time into sea water was higher. Such results could be explained by the fact that the UV radiation differentially weathered plastic when in wet or dry conditions. Moreover, these results could suggest that photooxidation products left the PE surface and were released into the liquid fraction leading to the observation of a lowly altered polymer surface by FTIR analysis (Kane and Clare, 2019; Liu et al., 2022; Tang et al., 2024; Ghatge et al., 2020; Montazer et al., 2020). Indeed, according to literature reports, plastic aging artificially induced via UV light exposure led to the formation of monomers, oligomers, and short chain compounds as degradation products (Kane and Clare, 2019; Liu et al., 2022; Tang et al., 2024). Specifically, the photoaging of PE for 5 days or 2000h resulted in the production of a series of carboxylic acids (CxH2x−2O4; x = 8–20) (Ghatge et al., 2020; Montazer et al., 2020).

3.2. Bacteria-mediated untreated ^{13}C polyethylene mineralization

We initially conducted assays with untreated ^{13}C-PE with 0.5 g/L of yeast extract (ye) as co-nutrient source by using *B. velezensis* MT9, *V. titanicae* MT11 and *V. venusta* MT1 isolates. No δ^{13}C-values increments were observed in the *Vreelandella* sp. bacterial cultures *i.e.*, MT11 and MT1, compared to the respective sterile ones (UNT ^{13}C-PE + ye). Furthermore, the *Vreelandella* sp. cultures, whether supplemented with ^{13}C-PE or not, exhibited the same bacterial growth trend, indicating that the polymer did not enhance bacterial growth, and this is consistent with the absence of detectable mineralization activity (data not shown).

On the contrary, δ^{13}C-values slightly increased in the *Bacillus* strain (MT9) cultures during the first 7 days, after which they levelled off (Goudriaan et al., 2023; Vaksmaa et al., 2023). These results suggest a low mineralization activity for untreated PE (Fig. 2.) or that most bacterial cells turned into spores that do not exhibit biodegradation activity towards the polymer. Another explanation could be that the mineralization dealt with residual low-molecular-weight carbon fractions, such as oligomers or short-chain hydrocarbons, that might be present due to incomplete polymerization during the synthesis of the labelled PE. Moreover, the ^{13}C excess production (µmol), calculated from the fractional abundance ^{13}C (^{13}F) (δ^{13}C-values for each time point in the total inorganic carbon pool ΣCO_2 (DIC + HS), as mentioned in the material and methods), is reported in Fig. 3 up to 60 days incubation. The mineralization of untreated ^{13}C-PE was only observed in the *Bacillus* strain cultures. The net $\Delta\ \delta^{13}$C (δ^{13}C *Bacillus* - δ^{13}C abiotic; $p < 0.045$) at the end of the incubation (140 days) was 2.59 ± 2.23 ‰, that corresponds to a $^{13}C_{net}$ excess of 0.0161 ± 0.0081 µmol of the total amount of CO_2 per incubation bottle (ΣCO_2; 653.18 µmol). This corresponded to a ^{13}C-mass loss % of 0.036 ± 0.021 with respect to the initial amount of polymer added *i.e.*, 1 mg (Table 1). For the abiotic control, the ΣCO_2 was markedly lower (200.74 µmol) compared to the ones achieved in the *Bacillus* sp. cultures (653.18 µmol), where PE was partially used, along with yeast extract, as carbon and energy sources. In our previous work (Bajo et al., 2024), we demonstrated a significant LLDPE film biodegradation ability of the same *Bacillus* strain MT9 via gravimetric weight loss measurement, FTIR analysis and SEM observations. Formerly, a mass loss of the untreated LLDPE film of 2.597 ± 0.971 % was measured after 60 days incubation. In the present work, after a longer incubation time (140 days), lower biomineralization activities toward untreated PE (0.036 ± 0.021 %) was observed with the same culture and no activities were detected in the presence of the other strains. The discrepancy between the results mentioned can be ascribed to: a) the PE type utilized in stable isotope assays was sold as middle/high-density PE by Sigma-Aldrich, whereas in previous work, we dealt with LLDPE that is less resistant than MDPE/HDPE and more susceptible to biodegradation (Ghatge et al., 2020; Satlewal et al., 2008), b) microbial degradation is associated with surface erosion in the polymer, consequently, the surface area to volume ratio is a crucial factor (more cells facilitate higher degradation rates) (Bitalac et al., 2023). The specific surface area of PE-MPs particles (a mix of films, spheres, and fragments around 100 µm in diameter) was reported as 179 cm^2/g by Rozman et al. (2023). We used 1 mg of ^{13}C-PE powder in the ^{13}C-stable isotope assay, this corresponds to an estimated surface area of 0.179 cm^2. In contrast, the LLDPE film used for previous assay had a surface area of 4.5 cm^2, which may explain the lower mass loss recorded in the ^{13}C-isotope labelled assay, c) the different incubation setups, *i.e.*, serum bottles (^{13}C-isotope labelled assay) and shake flasks (LLDPE assays), may also affect oxygen and nutrient availability, thereby influencing the extent of biotic degradation of PE and d) bacteria mediated CO_2 production directly from the marked PE was measured, thus, plastic biofragmentation processes, that could lead to an overestimation of the plastic-mass loss, are not taken into account (Montazer et al., 2020; Sardar, 2025).

3.3. Bacillus-mediated UV-treated ^{13}C polyethylene mineralization

Given the significant biodegradation potential expressed by the *Bacillus* sp. isolate MT9, the latter was used to test the effect of UV treatment on the final mineralization of the ^{13}C-PE under the same incubation conditions in presence and absence of yeast extract. In presence of yeast extract, the δ^{13}C value increased from 2.95 ± 0.56 ‰ at time zero to 14.8 ± 0.53 ‰ at the 7th days incubation after which it slowly incremented reaching 17.94 ± 1.51 ‰ after 60 days (Fig. 2.). A similar trend was observed in the parallel active cultures without yeast extract. Nevertheless, δ^{13}C values increased more slowly than in the presence of yeast extract and levelled off around 15.91 ± 0.46 ‰ since the 14th day. During the same time interval, the δ^{13}C values in the parallel sterile cultures with ($p < 0.0005$) and without ($p < 0.0001$)

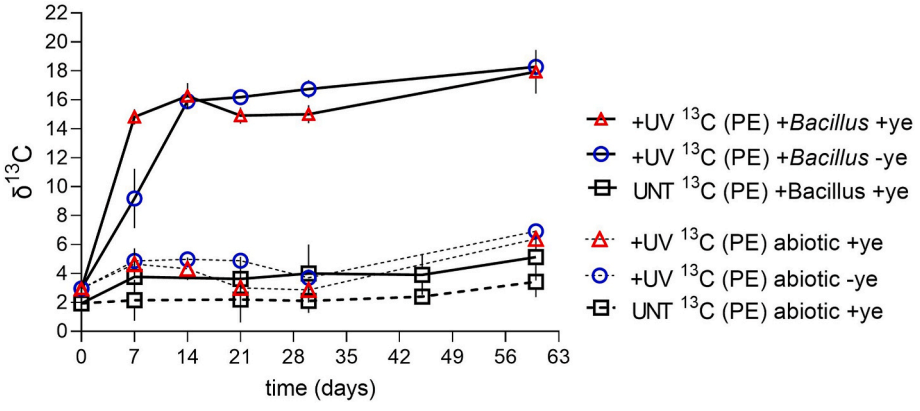

Fig. 2. Changes in δ^{13}C values during incubations with untreated ^{13}C-PE and ye, UV-treated ^{13}C-PE with ye, and UV-treated ^{13}C-PE without ye for *B. velezensis* isolate MT9 and uninoculated controls (abiotic).

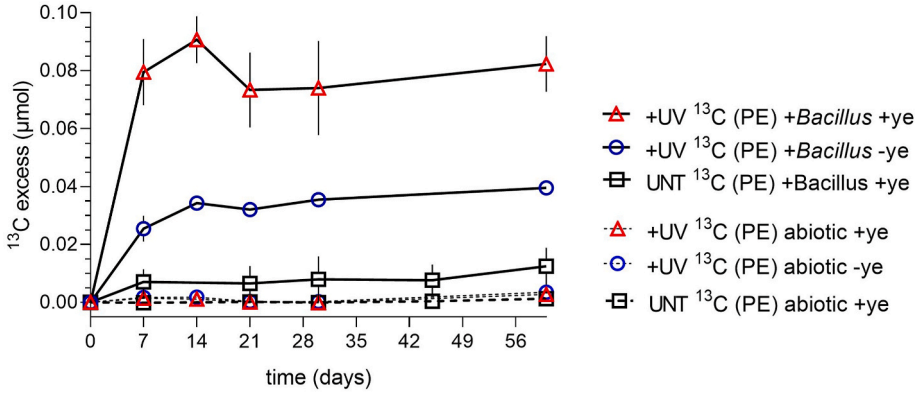

Fig. 3. Changes in ^{13}C excess (µmol) during incubations with untreated ^{13}C-PE and ye, UV-treated ^{13}C-PE with ye, and UV-treated ^{13}C-PE without ye for *B. velezensis* isolates MT9 and uninoculated controls (abiotic).

Table 1
Changes in^{13}C mass loss % during *B. velezensis* MT9 incubation with untreated^{13}C-PE and yeast extract, UV-treated^{13}C-PE with yeast extract and UV-treated^{13}C-PE without yeast extract for up to 140 days and extrapolation over a year. (*n.a.: not available*).

Time (day)	^{13}C-mass loss % +UV(^{13}PE) + ye	^{13}C-mass loss % +UV(^{13}PE) -ye	^{13}C-mass loss % +UNT(^{13}PE) + ye
0	0	0	0
7	0.181 ± 0.026	0.062 ± 0.013	0.019 ± 0.013
14	0.199 ± 0.016	0.084 ± 0.001	n.a.
21	0.171 ± 0.033	0.086 ± 0.005	0.025 ± 0.015
30	0.172 ± 0.039	0.092 ± 0.005	0.029 ± 0.019
45	n.a.	n.a.	0.023 ± 0.014
60	0.184 ± 0.022	0.094 ± 0.003	0.029 ± 0.019
90	n.a.	n.a.	0.034 ± 0.022
140	n.a.	n.a.	0.036 ± 0.021
365 (extrapolated)	0.737	0.462	0.079

yeast extract, were significantly lower compared to their corresponding biotic incubations. Thus, *Bacillus* sp. isolate MT9 significantly mineralizes ^{13}C-PE and yeast extract accelerates the process by sustaining bacterial growth at the beginning of incubation i.e. mainly in the first 7 days. Indeed, the bacterial planktonic bacterial cell counts (Fig. 4.) were found to mainly increase in the first 7–14 days incubation in the presence of yeast extract and then remain constant up to the end of the incubation. On the other hand, in the absence of yeast extract, a drop of the planktonic bacterial cell counts was observed in the first 7 days that was followed by an increase of microbial cell concentration up to 14 days and then the cell counts remained constant until the end of the experiment. These results, suggest that from day 14 most bacterial cells turned into spores with no biodegradative activity towards UV-treated PE. These results are consistent with those collected in previous studies (Goudriaan et al., 2023). The µmol of ^{13}C excess production resulting from fractional abundance ^{13}C (^{13}F) based on δ^{13}C-values and the µmol of ΣCO_2 (DIC + HS) was also determined (Fig. 3.). The δ^{13}C-values were comparable in magnitude and trend in the presence and absence of yeast extract. Nevertheless, the total amount of CO_2 produced (ΣCO_2) was much higher with yeast extract (940.95 µmol) than without it (359.46 µmol). This could be attributed to the yeast extract metabolism that led to the accumulation of $^{12}CO_2$ and dilutes the $^{13}CO_2$ from the mineralization of the ^{13}C-PE. The $^{13}C_{net}$ excess productions (µmol) of 0.072 ± 0.008 for the UV-treated ^{13}C-PE with yeast extract and of 0.036 ± 0.001 for UV-treated ^{13}C-PE without yeast (Fig. 3.) correspond to ^{13}C-mass losses % of 0.184 ± 0.022 and 0.094 ± 0.003, respectively (Table 1).

Overall, based on the results of UV-treated PE degradation, the fact

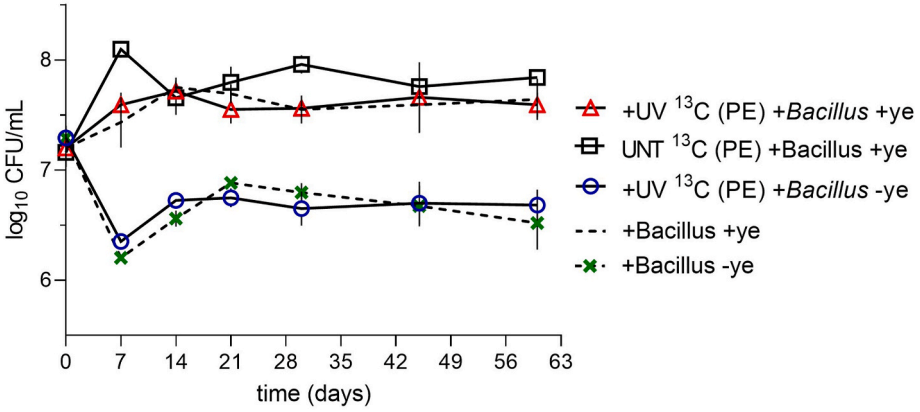

Fig. 4. Evaluation of *B. velezensis* MT9 growth ($\log_{10}$ CFU/mL) in mineral medium with untreated ^{13}C-PE + ye, UV-treated ^{13}C-PE + ye and UV-treated ^{13}C-PE-ye. Furthermore, the *B. velezensis* MT9 was cultured in ^{13}C (PE)-free mineral medium (biotic control), both with and without ye.

that ^{13}Cex reached a plateau phase starting from 14 days incubation may be explained by one/a combination of the following factors: i) the bacterial cells were in sporulation phase and the spores are not able to attack the polymer, ii) limitations in bioavailable carbon i.e. *Bacillus* sp cells were able to degrade mainly photooxidation products released from the PE after UV treatment such as nanoplastics, oligomers, monomers and short-chain compounds that have functional groups making them easier to attack by microbial cells/enzymes (Dhanraj et al., 2025; Goudriaan et al., 2023; Romera-Castillo et al., 2018; Wayman and Niemann, 2021; Zheng et al., 2022) iii) the growth of *B. velezensis* in biofilm (Liu et al., 2022) covering the surface of all available surface on PE particles and limiting the transport of nutrients inside the biofilm which could have led to a reduction of microbial activity and consequently to a constant transformation rate of ^{13}C-PE to ^{13}C-CO_2.

Our research highlights that *Bacillus velezensis* MT9 is able to mineralize PE. Even though the mineralization of PE in our study is low, the observed rates are in line with the results of the few available research reports on PE mineralization (Table 2). Indeed, although it is very challenging to compare the results of the mineralization kinetics we obtained here with literature reports, due to different types of polymers, microbes and experimental conditions used; we report a highest mineralization rate of UV-treated PE of 0.026%/day (0.181%/7days) in the presence of yeast extract in the first 7 days incubation which is in the same order of magnitude as the mineralization rates reported for other bacteria and fungi. Specifically, Goudriaan et al., 2023 reported mineralization rates of up to 0.105%/month i.e. 0.0035%/day for *Rhodococcus ruber* (Goudriaan et al., 2023); while Taipale and colleagues reported mineralization rates of up to 0.15%/month using SIP-based assays to trace ^{13}C-label from PE in a lacustrine food chain (Taipale et al., 2023). Also, a PE mineralization rate of 0.01%/day was observed by the marine yeast *Rhodotorula mucilaginosa* (Vaksmaa et al., 2023) and, for UV-treated PE, a mineralization rate of 0.044%/day by the marine mould *Parengyodontium album* was reported by (Vaksmaa et al., 2024).

The description of PE biodegradation mechanism by isolate MT9 based only on the results of PE mineralization obtained here remains speculative. Nevertheless, taking into consideration the available literature reports describing bacterial mechanisms of PE degradation, the fact that *B. velezensis* isolates have been reported to produce laccases and peroxidases and the findings of our previous study, a hypothetical PE biodegradation mechanism has been proposed in our previous work for the isolate MT9 (Bajo et al., 2024). Such proposed mechanism might involve in a first step, the non-specific oxidation of the polymer through the production of extracellular reactive oxygen species. Then, extracellular enzymes such as peroxidases, oxygenases, and/or laccases are involved in breaking PE into smaller fragments and introduction of carbonyl groups. These smaller molecules are then transported into the microbial cells and further degraded by enzymes like hydroxylases, monooxygenases, and lipases, following pathways similar to those exploited by the cells for alkanes biodegradation/mineralization (Gao et al., 2022; Rong et al., 2024a, 2024b; Zadjelovic et al., 2022; X. Chen et al., 2025; Dhanraj et al., 2025; Sardar, 2025; Trad et al., 2025). In the case of *B. velezensis* C5, based on genome sequencing and in silico analysis, Liu et al. (2022) hypothesised that laccase, propionyl-CoA carboxylase and cytochrome P450 (CYP) enzymes might play key roles in PE degradation by the isolate (Liu et al., 2022). The same isolate has been reported to exhibit a high biodegradation rate of PE subjected to cobalt-catalysed carbonyl functionalization and to show a laccase activity 25 times higher compared to the control, supporting involvement of the enzyme in PE oxidation (Tang et al., 2024). Further studies including genomic and/or transcriptomic analyses are required in order to be able to elucidate PE biodegradation mechanisms by *B. velezensis* isolate MT9.

Table 2
Comparison of the^{13}C-mass loss rate of labelled PE among the very few reports available in the literature.

Micororganism	^{13}C-mass loss rate	References
Bacillus velezensis MT9	0.181%/7days	this study
Rhodococcus ruber	0.105 %/month	Goudriaan et al. (2023)
Rhodotorula mucilaginosa	0.01%/day	Vaksmaa et al. (2023)
Uncultured Acetobacteraceae* and *Comamonadaceae	0.15/month	Taipale et al. (2023)
Parengyodontium album	0.044%/day	Vaksmaa et al. (2024)

3.4. Extrapolation of Bacillus-mediated biomineralization rate per year

Bacteria have been reported to more promptly degrade photooxidation products of PE than the virgin PE (Montazer et al., 2019). It is reported that under life-threatening conditions (lack of nutrients such as carbon, nitrogen, and phosphorus), many *Bacillus* species can survive by forming endospore (Christov et al., 2018). The bacterial concentration was in this study measured using vital cell-count method on a nutrient-rich solid medium (Bajo et al., 2024) which does not discriminate between vegetative or endospore forms (Christov et al., 2018). To address this limitation, the Shaeffer-Fulton staining method (Hussey, 2013) was used for endospore detection in the second part of the incubation, when a stable mineralization rate plateau was achieved, numerous endospores depicted in bright green were observed in all active cultures (Fig. 2S.).

This suggests that *Bacillus* cells, due to nutrient imbalance, would pass from a vegetative to an endospore form. This would probably be responsible for the plateau in the mineralization rate in the ^{13}C-PE *Bacillus sp* - culture after 30 days of incubation. The isotope labelling tracing technique allows to measure plastic degradation rates in much shorter time periods (days to weeks) than commonly used method (months to years). Nevertheless, one has to be wary of extrapolating the ^{13}C-mass loss calculated for brief incubations (days) and translating it to much longer periods (years) even though the thought is tempting. This way, one would assume first-order kinetics and a constant active bacterial concentration throughout the entire experimental incubation. Therefore, the tracing isotope assays were extended to 60 days (UV-treated assays) and 140 days (untreated assays) with respect to what has been reported previously (incubation from 5 (Vaksmaa et al., 2023) to 30 (Goudriaan et al., 2023) days) for a comprehensive examination of the mineralization process.

The prolongation of the incubation period to 60 or 140 days did not result in a net increment of ^{13}C mass loss % (Table 1) probably for the *Bacillus* cell sporulation. Indeed, the *Bacillus* cells turned into spores as was observed by light microscopy (Fig. S2). These results indicate that PE degradation under nutrient-poor conditions may be self-limiting and cell sporulation complicates the modelling of long-term environmental degradation. In natural settings, fluctuating nutrient availability and the gradual release of degradation products are likely to create a dynamic system in which microbial cells alternate between vegetative growth and sporulation in response to nutrient balance, influencing degradation rates. Nevertheless, we extrapolated the mineralization rate per year but calculating the slope (Δ^{13}C-mass loss % · Δday^{-1}) over a much longer time interval than what was done before (Goudriaan et al., 2023; Vaksmaa et al., 2023). Thus, the ^{13}C-mass loss was up to 0.079, 0.462, and 0.737 % yr^{-1} for *Bacillus* incubated with untreated ^{13}C-PE with yeast extract, UV-treated ^{13}C-PE without yeast extract, and UV-treated ^{13}C-PE with yeast extract, respectively. The mineralization of PE has been assessed in the presence of *Rhodococcus ruber* strain C208 (Goudriaan et al., 2023), of the marine yeast *Rhodotorula mucilaginosa* (Vaksmaa et al., 2023) isolated from a 350 L laboratory seawater microcosm contaminated with various plastics (Vaksmaa et al., 2023) and the marine mould *Parengyodontium album* (Vaksmaa et al., 2024).

All of these studies employed stable isotope tracing assays with ^{13}C-labelled polyethylene. The analyses revealed polymer mineralization rate up to 1.2% yr^{-1}; 3.8% yr^{-1} and 0.044% day^{-1} for *Rhodococcus*, *Rhodotorula* and *Parengyodontium,* respectively.

Our mineralization results, along with those aforesaid, pose challenges in comparison with traditional plastic biodegradation assessments such as gravimetric weight loss due to their lower sensitivity. Our methodological approach allows to measure δ^{13}C values of approximately 1‰ with confidence, translating to detectable ^{13}C-mass changes of the ΣCO_2 pool of 0.010%, 0.006%, and 0.015% for untreated ^{13}C-PE with yeast extract (140 days), UV-treated ^{13}C-PE without yeast extract (60 days), and UV-treated ^{13}C-PE with yeast extract (60 days), respectively. This detection limit is influenced by the background CO_2 levels (0.34 mmol without yeast extract and 0.65–0.94 mmol with yeast extract) and the added ^{13}C-PE (1 mg). The ΣCO_2 pool's variance impacts the detection limit, with higher ΣCO_2 levels in the presence of yeast extract increasing the detection limit due to ^{13}C dilution from yeast extract metabolism. As a result, our method is orders of magnitude more sensitive than commonly used techniques. Caution is required even when comparing our results with other mineralization data, as factors such as incubation condition (temperature, shaking, *etc*.) and surface-to-volume ratio can drastically influence the mineralization rate. Nevertheless, our sensitivity is comparable to other studies using stable isotope assays, which report detection limits in the range of 0.002–0.004%, achieved with lower ΣCO_2 levels but similar amounts of ^{13}C-PE compared to our study. For example, using a similar experimental setup to that applied for evaluating PE degradation and assimilation by the marine yeast *Rhodotorula mucilaginosa*, where the detection limit for polyethylene degradation was approximately 0.002% of the added polyethylene (~1.8 and ~1.7 mg), our method enables the detection of plastic degradation at 0.006% for UV-treated ^{13}C-PE without yeast extract. Hence the detection limits are comparable and our method demonstrates a sensitivity within the same magnitude as the other authors who utilized IRMS (Vaksmaa et al., 2023).

Additionally, the previously mentioned possibility of released photooxidation products in the liquid phase may serve as an easy carbon source that would explain the high degradation rates observed in the beginning of the incubation. Under environmental conditions, weathering would occur at slower rates, presumably under slow but constant release of these products, limiting degradation under these conditions. Also, in real-world scenarios, the mineralization of PE is influenced by a variety of environmental factors that are not fully replicable under controlled laboratory settings. Indeed, in our work we performed the experiments attempting to simulate some of the factors such as salinity (we used a mineral medium having 30 g/l of NaCL) and photooxidation (the UV dose we used is equal to approximately 50 days of radiation at the sea surface in subtropical oceans or ~125 days in temperate marine regions). However, other factors including temperature/pH fluctuations, oxygen and nutrient availability, wave currents, humidity, and the presence of a diverse microbial community can impact the biodegradation/mineralization process. Nevertheless, this does not necessarily imply that biodegradation rates in real marine environment, where the conditions are not optimized for the degradation/mineralization of the polymer, would be much higher or that the biodegradation would be quicker compared to what we observed in our study under lab scenario and using a pure bacterial culture (with optimal conditions for bacterial growth and activity). Indeed, we performed a deep literature review on plastics mineralization under real marine environmental conditions and the findings are that a low biodegradation was observed under in-situ conditions (Sun et al., 2025). Such biodegradation has been evaluated based on methods other than monitoring labelled PE mineralization. Nauendorf et al. (2016) reported that no evidence of biodegradation of TiO2-commercial polyethylene carrier bags was recorded after incubation for 98 days in natural oxic and anoxic sediments from the Western Baltic Sea (Nauendorf et al., 2016). Sudhakar et al. (2007) reported maximum gravimetric weight losses of up to 2.5% and 0.8% for commercial LDPE and HDPE after 6 months in-situ incubation in ocean waters (Sudhakar et al., 2007). After 20 months incubation at a depth of 2 m in the Baltic Sea, no evidence of polyethylene-starch blends biodegradation was found (Rutkowska et al., 2002). Brümmer et al. (2022) reported that polyethylene microplastics sampled from the inside of a ship that was foundered twenty-nine years before at the coast in the red sea showed no signs of degradation, while beached microplastics exhibited structural changes and fragmentation as a result of exposure to photooxidation and high temperature on beach environment; confirming the low degradation rate of PE in seawater and the important role of temperature and photooxidation in (bio)degradation process of PE (Brümmer et al., 2022).

4. Conclusions

Our results demonstrate that marine *Bacillus velezensis* MT9 can gradually mineralizes PE plastics. Incubation with UV-treated ^{13}C-PE resulted in a significantly higher ^{13}C-mass loss % compared to untreated plastic. Therefore, plastic photooxidation is a crucial process required in order to make the plastics susceptible to microbial biodegradation (Goudriaan et al., 2023; Romera-Castillo et al., 2018; Vaksmaa et al., 2023, 2024; Wayman and Niemann, 2021). Additionally, the MT9 can utilize more efficiently PE for energy provision in the presence of other substrates (i.e. yeast extract) indicating that bacterial plastic degradation can indeed occur in the natural environment, although at low rates. Nevertheless, existing mineralization studies are scarce, and all indicate low degradation rates due to the intrinsic recalcitrance of polyethylene. This may be attributed to the recent plastics pollution phenomenon, and this did not allow bacteria to adapt and produce enzymes specifically targeting the loose polymers of plastics, especially polyolefin based on a C-C backbone. Overall, the biological degradation of polyethylene should be regarded a long-term natural attenuation mechanism operating over extended environmental timescales.

Hence, a prospective approach for the future could be not only selecting bacteria from contaminated sites but also accelerating their adaptation in lab-scale experiments for the discovery of novel/engineered enzymes involved in PE biodegradation.

CRediT authorship contribution statement

Kejvin Bajo: Writing – original draft, Methodology, Investigation. **Boris Kolvenbach:** Writing – review & editing, Validation, Supervision. **Philippe F.-X. Corvini:** Writing – review & editing, Resources, Conceptualization. **Fabio Fava:** Writing – review & editing, Resources. **Noura Raddadi:** Writing – review & editing, Supervision, Conceptualization.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ibiod.2026.106294.

Data availability

Data will be made available on request.

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