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RESEARCH

Biodegradation of plastics by the phosphorus-solubilising cyanobacterium *Chroococcus* sp.

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Abstract

Plastics often contain additives such as flame retardants, plasticisers, and stabilisers, with phosphorus-based compounds playing a key role in microbial interactions. Most previous studies have focused on heterotrophic microbes targeting carbon backbones, but their degradation efficiency remains limited. This study examined the phosphorus-solubilising ability of autotrophic cyanobacteria carrying the *phoD* gene to weaken polypropylene (PP) and polyethylene terephthalate (PET). Plastic samples collected from the Aquatic Research Complex fishpond at Universiti Sains Malaysia were incubated with isolates and analysed using NBRIP medium, molybdenum blue assay, UV–Vis, FTIR, SEM, and tensile strength testing over 61 days. Of the five strains isolated, *Chroococcus* sp. solubilised phosphorus from both PP and PET, releasing phosphorus into the medium and reducing plastic surface phosphorus. Treated plastics exhibited surface roughness, reduced dry mass, and significant tensile strength loss. This is the first report of photosynthetic cyanobacteria promoting plastic degradation via phosphorus scavenging.

Keywords Phosphorus-solubilising microbes · Cyanobacteria · Plastic degradation · Polypropylene · Polyethylene terephthalate

Introduction

Plastics are not only carbon-based polymers but also contain a wide range of additives that enhance their performance during processing, such as injection moulding and extrusion (Andrady and Neal 2009; Thompson et al. 2009). These include flame retardants, plasticisers, stabilisers, pigments, lubricants, and antistatic agents (Geyer et al. 2017; Hahladakis et al. 2018). Soluble additives such as di(2-ethylhexyl) phthalate (DEHP) and bis(2-ethylhexyl) adipate (DEHA) improve polymer flexibility (Koch and Calafat 2009), while sodium lauryl sulfate (SLS) enhances wetting and adhesion (Reemtsma et al. 2008). Phosphoric acid esters such as tris(2-ethylhexyl) phosphate (TEHP) and tributyl phosphate (TBP) increase plastic processability (Van der Veen and de Boer 2012). Other phosphorus compounds, such as aminoalkylene phosphonates, function as flame retardants (Weil and Levchik 2004). Although insoluble additives such as bisphenol A (BPA) provide strength and clarity, they can leach into the environment (Vandenberg et al. 2007). Other examples of additives include antimony trioxide (Sb_2O_3), used in polyethylene terephthalate (PET), and titanium dioxide (TiO_2), a

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pigment and UV stabiliser (Weil and Levchik 2004). Organophosphorus and halogenated compounds, such as triphenyl phosphate (TPP) and resorcinol bis(diphenyl phosphate) (RDP), increase flame retardation (Laoutid et al. 2009). Together, these components increase the chemical complexity and resilience of plastics against degradation.

Most of the earlier research into microbial plastic degradation targeted the carbon backbone of plastics (Sivan 2011). For instance, the bacterium *Ideonella sakaiensis* depolymerises PET with PETase and MHETase into terephthalic acid (TPA) and ethylene glycol (EG) to metabolise them (Yoshida et al. 2016; Austin et al. 2018). EG can be further metabolised by *Pseudomonas putida* (Franden et al. 2018). Poor activity has been shown from *I. sakaiensis* for highly crystalline PET (Tournier et al. 2020). Generally, carbon assimilation pathways' effectiveness is inhibited by the strength and additive complexity of plastics (Gewert et al. 2015; Urbanek et al. 2018). Because of the stable polymer structures and embedded additives that substantially slow degradation, large-scale application is not possible (Shah et al. 2008). Targeting non-carbon additives can be an alternative approach. According to Danso et al. (2019), while additives enhance the performance of plastics, microbial degradation of such additives could weaken the matrix and expose the carbon chains to further degradation. Additive assimilation may, therefore, foster microbial colonisation and plastic fragmentation, thus providing points of entry for both autotrophic and heterotrophic organisms.

According to Zettler et al. (2013), many environmental plastic surfaces are covered by biofilms dominated by autotrophs, including cyanobacteria and algae. The growth of these organisms depends on sunlight and CO_2, and thus, they do not rely on carbon from the plastic (Paerl et al. 2011). However, they require essential nutrients such as phosphorus, which is generally employed as a plastic additive (Elser et al. 2007; Rummel et al. 2017). This non-carbon nutrient requirement creates the path for a two-step degradation. First, by degrading additives with autotrophic organisms, hence compromising structural integrity, and secondly, by exploiting heterotrophic microorganisms to further degrade it.

This work aimed to establish whether phosphorus-solubilising cyanobacteria are capable of improving the plastic degradation rate. The research was conducted by isolation and identification of the cyanobacterial strain, followed by the detection of the phosphorus-solubilising *phoD* gene. We then assessed the ability of the *phoD*-positive gene strain to solubilise phosphorus from insoluble sources. Solubilised phosphorus concentration was measured using the molybdenum blue assay at the beginning and end of the 61-day experiment using the National Botanical Research Institute's Phosphate (NBRIP) medium, which contained polypropylene (PP) and PET plastics. Fourier-transform infrared spectroscopy (FTIR) was used to analyse changes in chemical properties, and scanning electron microscopy (SEM) of surface morphology and dry mass loss were used to evaluate biodegradability. Lastly, mechanical integrity was assessed both before and after incubation using tensile strength testing.

Methodology

Sample collection, cyanobacterial isolation, and DNA extraction

Plastic samples were collected from the area around Universiti Sains Malaysia's Aquatic Research Complex Fishpond (coordinates: 5.358533, 100.293842). The samples included plastic waste products with green microbiological growth, suspected to be cyanobacteria, visible on their surfaces. These samples were placed in a 250 mL Erlenmeyer flask, shaken at 80 rpm, and incubated for a month with 24-h illumination (50 µmol photons $m^{-1}s^{-2}$) in BG-11 liquid medium (Rippka et al. 1979; Waterbury and Willey 1988) for microbial enrichment. To promote the growth and to screen the microbial colonies, the enriched cultures were transferred onto BG-11 agar plates after the incubation period (Stanier et al. 1971; Rippka et al. 1979). Distinct colonies were isolated based on their morphology. The strains were repeatedly sub cultured for purification and then propagated and extracted from the purified isolate. The media used was the Vivantis GF-1 Bacterial DNA Extraction Kit from Vivantis Technologies Sdn. Bhd., Selangor, Malaysia (Pattiram et al. 2014).

Detection of phosphorus-solubilising (*phoD*) gene

The genomic DNA purified for use in PCR was stored at −20 °C according to Lorenz (2012). All PCRs were performed using Vivantis Master Mix with Taq polymerase, dNTPs, $MgCl_2$, and reaction buffers. The following components were added to the PCR reaction mixture: 12.5 µL Taq polymerase, 1 µL forward primer, 1 µL reverse primer, 8.5 µL autoclaved H_2O, and 2 µL DNA template, for a total volume of 25 µL.

The *phoD* gene was amplified from the DNA samples by PCR according to the method of Fraser et al. (2015). The 500 bp *phoD* fragment was amplified through an optimised PCR procedure with the help of the primers *phoD* F: 5′-CAG TGG GAC GAC CAC GAG GT-3′ and *phoD* R: 5′-GAG GCC GAT CCG CAT GTC G-3′ (Sakurai et al. 2008; Tan et al. 2013). The first cycle started with an initial denaturation at 95 °C for 4 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 57 °C for 30 s, and extension at 72 °C for 30 s, and ended with an extension at 72 °C for 5 min (Sakurai et al. 2008; Tan et al. 2013). The results of the PCR were viewed by 1.5% agarose gel

electrophoresis, run at 70 V and 400 A for 40 min, according to Lee et al. (2012) and Sanderson et al. (2014). The gel, was stained in SYBR Safe DNA Gel Stain in TAE buffer and visualised by a UV gel documentation system.

Identification of phoD-positive gene species by 16S rRNA sequencing, MEGA software, and BLAST NCBI website

After detection of the potential phosphorus-solubilising (*phoD*) gene targeting a 500 bp region by assessing the fragment size from the gel UV samples, the next step involved the identification of microbes using 16S rRNA. Cyanobacterial identification was by sequencing the 16S rRNA gene using the primers CYA106F (5' CGG ACG GGT GAG TAA CGC GTG A 3') and CYA781R (5' GAC TAC TGG GGT ATC TAA TCC CAT T 3') (Nübel et al. 1997). The thermocycling conditions started with an initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation for 1 min at 94 °C, annealing for 1 min at 55 °C, and extension for 1 min at 72 °C, with a final extension step for 5 min at 72 °C, following the method by Nübel et al. (1997) and Taton et al. (2003). The PCR products were purified and forwarded for sequencing to NHK Bioscience Solution SDN. BHD, Malaysia. Sequences were checked using Molecular Evolutionary Genetics Analysis (MEGA), Version 6.0, according to the procedure by Tamura et al. (2013). A BLAST search was performed following the procedures outlined by Altschul et al. (1990) and Stackebrandt and Goebel (1994) to determine the similarity of sequences to known genes in the NCBI database.

Assessment of phosphorus-solubilising activity on sodium phytate, PP, and PET using NBRIP agar

The potential of phosphorus-solubilising microbes isolated from PP and PET plastics was analysed using the NBRIP medium developed at the National Botanical Research Institute. The plastic materials were thoroughly rinsed with distilled water, followed by sterilization using 70% ethanol rinsing, UV radiation, and then drying under sterile conditions before use. This step was performed to eliminate potential contaminants that could interfere with microbial activity during the incubation period. The NBRIP medium was prepared by incorporating 10 g L^{-1} glucose, 5 g L^{-1} $MgCl_2.7H_2O$, 0.25 g L^{-1} $MgSO_4.7H_2O$, 0.2 g L^{-1} KCl, 0.1 g L^{-1} $(NH_4)_2SO_4$ and an insoluble phosphate source, such as 5 g L^{-1} $C_6H_6Na_{12}O_{24}P_6$. Additionally, insoluble phosphate was obtained from the PET and PP plastics. The medium was further supplemented with trace elements, including 10 g L^{-1} EDTA and 2.2 g L^{-1} $MnSO_4.H_2O$, 1 g L^{-1} $FeSO_4.7H_2O$, 0.5 g L^{-1} $CuSO_4.5H_2O$, 0.3 g L^{-1} $CoCl_2.6H_2O$, 0.2 g L^{-1} $Na_2MoO_4.2H_2O$, and 0.1 g L^{-1} $CaCl_2$ (Illmer and Schinner 1992; Wan et al. 2020). The medium was autoclaved at 121 °C for 15 min at 15 psi. The NBRIP agar medium was then aseptically inoculated with phosphorus-solubilising microbes and incubated at 30 °C for 7 days, according to Bashan et al. (2013) and Halder et al. (1990). The clear zones surrounding the microbial colonies after incubation indicated successful phosphate solubilisation.

Assessment of soluble P in NBRIP by PP and PET using molybdenum blue reagent and UV–Vis spectroscopy

We measured the concentration of soluble phosphorus in the NBRIP liquid medium containing PP and PET polymers both before and after a 61-day incubation period using a VIS spectrophotometer and the molybdenum blue reagent. PP and PET plastics were used as the sole sources of phosphorus. At the same time, the samples were cultured in an NBRIP minimum liquid medium (H_2O); 5 g of ammonium molybdate was dissolved in 100 mL of distilled water to create an acidified molybdate solution. Then, 25 mL of concentrated sulphuric acid was added slowly while stirring vigorously (Murphy and Riley 1962). 0.2 g of potassium antimonyl tartrate $K(SbO)C_4H_4O_6.½H_2O$ was dissolved in 100 mL distilled water to make the antimonyl tartrate solution (Wei and Zimmermann 2017). One gram of ascorbic acid was first dissolved in 100 mL of distilled water to make a fresh solution of ascorbic acid. To obtain the final molybdenum blue reagent, 10 mL ammonium molybdate solution and 5 mL potassium antimonyl tartrate solution were dissolved in 85 mL distilled water. This cocktail of reagents was carefully mixed with 5 mL of freshly prepared ascorbic acid solution according to the method by Wei and Zimmermann (2017). The absorbance at 880 nm was measured when the blue colour developed at 10 min after the mixture had been incubated at room temperature (Arias-Andres et al. 2018). A standard curve with a known phosphate concentration was plotted to determine the amount of solubilised phosphorus present in the samples. The microbial ability to solubilise phosphate was assessed by comparing the phosphorus concentrations in the inoculated samples with those in an uninoculated control.

Assessment of phosphorus band magnitude in PP and PET before and after 61-day incubation using FTIR

The phosphorus content in PP and PET mixed plastic was measured by FTIR ATR Perkin Elmer System 2000 at the School of Industrial Technology, Universiti Sains Malaysia. Measurement before and after 61 days of incubation in NBRIP liquid medium was made. Plastic samples with a size of 500 µm were sterilized and incubated in NBRIP liquid medium for 24 h at 28 °C, agitated at 80 rpm, and

illuminated by an LED at 50 µmol photons $m^{-1}s^{-2}$ (Waterbury and Willey 1988; Jung et al. 2018). After 61 days, plastic samples were taken out from the medium and cleansed thoroughly with distilled water to remove the remaining medium and microbial biomass. Cleaned polymers were left to dry at room temperature to avoid interference by moisture during the FTIR analysis.

An ATR-FTIR spectrometer with a resolution of 4 cm^{-1} and 32 scans per sample was used for the FTIR analysis covering the range from 4000 to 400 cm^{-1} according to the procedure of Kettner et al. (2017). After incubation, the changes in FTIR spectra were checked for peak intensity variations, appearance of new peaks, or shifting in already existing peaks, which would indicate microbial interactions, phosphorus binding, or possible breakdown of the plastic structure.

Assessment of degradation of PP and PET plastics after incubation

Chemical and physical characteristics of PP and PET plastic were analysed before and after 61 days of incubation in NBRIP liquid medium to evaluate their biodegradation. PET strips, with 15.000 mm width and 0.345 mm in thickness and a surface area of 5.175 mm^2, and PP strips, with 15.000 mm width and 0.130 mm in thickness and a surface area of 1.950 mm^2, were sterilised before being immersed in the liquid.

The experiments were carried out in triplicate for each condition in order to consider the potential mass loss due to microbial degradation. Before incubation, each plastic strip was measured for its initial dry mass. After incubation, the plastic strips were cleaned properly to remove residual medium. The strips were further washed with distilled water to remove the microbial biomass that loosely attached to the strips. Thereafter, the strips were air-dried at room temperature until the mass of the strips had stabilised. The mass loss for each replicate was measured separately and expressed as the mean standard deviation (SD) of the 3 replicates. This was obtained by determining the difference in mass as a way of calculating the degree of degradation and material loss. Scanning electron microscopy was used to observe the appearance of the plastic surface to find any structural changes that may be associated with microbial activity. SEM pictures were taken at magnifications of 800 × and 6000 × to capture possible changes such as surface roughness, microbial colonisation, erosion, or fissures that could indicate biodegradation.

Tensile strength evaluation of PP and PET plastics after incubation

The mechanical integrity of PP and PET strips was investigated by measuring tensile strengths before and after 61-day incubation in NBRIP liquid medium to evaluate the impact of phosphate-solubilising microbes on the structural strength of plastics.

The same plastic strips were not used for both pre- and post-incubation tests to ensure comparability and to avoid structural interference from previous testing. Instead, two identical sets of plastic strips were tested in triplicate (n = 3 per group). One set was used for the tensile strength testing control (before incubation), and the other set was incubated in medium to be tested later. Plastic strips were incubated for 61 days in NBRIP medium in a controlled environment.

After incubation, the plastic strips were cleaned thoroughly with distilled water to remove residual medium and microbiological debris and air-dried at room temperature to constant mass. The highest tensile force that each strip could bear prior to breaking was determined on a Universal Testing Machine (UTM) VantageNX Duo Tensile Tester 5 kN (1125 lbs) at the School of Industrial Technology, Universiti Sains Malaysia. The control and treatment groups' tensile strength values (measured in megapascals, or MPa) were then compared to assess any possible plastic deterioration or weakening brought on by microbial activity.

Results

Cyanobacterial identification

Five different microbial strains were isolated from the agar media. Only one strain tested was positive for the *phoD* alkaline phosphatase gene, according to molecular screening that targeted a 500 bp area of the gene. With a 16S ribosomal RNA gene similarity of 93.06% (sequence ID: KM019988.1), the BLAST search revealed the closest resemblance to the cyanobacterium *Chroococcus* sp.

Phosphorus solubilisation from sodium phytate, PP, and PET plastics

The *phoD*-positive microbial strain's capacity to solubilise phosphorus was evaluated using NBRIP medium, a minimum medium designed to measure microbial access to phosphorus from insoluble sources. Microbial phosphorus uptake was evaluated using two different experimental strategies. To determine microorganisms that could solubilise phosphorus, sodium phytate was used as the only phosphorus source. This test determines the microbes' capacity to hydrolyse sodium phytate into soluble phosphate forms, which was shown by the development of transparent halo zones surrounding microbial colonies during incubation. This stage confirmed whether phosphatase or phytase activity was present. The following test used plastic materials (such as PET and PP) that were placed in a solution devoid of phosphorus.

The purpose of these tests was to determine if the chosen microorganisms could directly scavenge phosphorus from the plastic matrix.

Figure 1 shows that the tested cyanobacterium strain generated a noticeable halo zone when cultivated in NBRIP medium containing sodium phytate. This demonstrated the strain's potential to increase phosphorus bioavailability in environmental settings by confirming its capacity to solubilise phosphorus. Next, sodium phytate in the NBRIP medium was replaced with PP or PET plastic to investigate whether the strain could access phosphorus from a synthetic polymer.

Cyanobacterial-mediated phosphorus release from PP and PET plastics in liquid medium

We studied the ability of the cyanobacterium strain to scavenge phosphorus from PP and PET plastics through prolonged incubation in NBRIP minimum media. As this medium has no phosphorus supply, any detectable phosphorus present in the system would either be from the plastic or the bacteria themselves (Nautiyal 1999). Upon the release of phosphorus to the liquid medium, microbes will take it up through high-affinity phosphate transport systems and incorporate it into key biomolecules, including ATP, phospholipids, and nucleotides (Santos-Beneit 2015). This is because the uptake of phosphorus is crucial for microbial survival and activity when degrading plastics, since it is one of the vital ingredients for energy transfer, cell membrane structure, and the creation of genetic material.

In an attempt to monitor the quantity of soluble phosphorus in the liquid medium during incubation, a reliable colorimetric technique frequently used in the research of phosphorus quantification, the molybdenum blue test, was adopted (Murphy and Riley 1962). The molybdenum blue method was employed for monitoring phosphorus release as a function of time by measuring absorbance at 880 nm (Fig. 2). These results show a gradual increase, during incubation time, of soluble phosphorus for both PP and PET plastics, which demonstrates the ability of the cyanobacterium strain to mobilise phosphorus from these materials. Therefore, these findings were interpreted as preliminary evidence supporting the potential role of the cyanobacterium in phosphorus scavenging from plastic waste.

Changes in phosphorus content by comparing band magnitude evaluated using ftiR

Fourier Transform Infrared Spectroscopy (FTIR) examination was performed on untreated PP and PET plastic to assess any changes in phosphorus content. The result confirmed a drop in the total phosphorus content by showing a decrease in the distinctive peaks linked to phosphorus-containing compounds (Fig. 3). These changes imply that either the direct consumption of phosphorus or its release via the disruption of phosphorus-associated chemical bonds within the plastic matrix was triggered by microbial activity.

Microbial-induced physical changes in PP and PET plastics

Subsequently, after the 61-day incubation with *Chroococcus* sp., changes in physical features of both PP and PET plastics and their chemical characteristics concerning phosphorus solubilisation were analysed. The pre- and post-incubation biomasses of PP and PET were analyzed statistically using paired t-tests. The mean PP biomass decreased from 0.0608 ± 0.0001 g before incubation to 0.0605 ± 0.0001 g after incubation (mean ± SD, $n = 3$), resulting in a mean loss of 0.00027 g. Similarly, the PET biomass decreased from 0.3095 ± 0.0001 g pre-incubation to 0.3092 ± 0.0001 g post-incubation, again resulting in a mean loss of 0.00027 g. According to statistical analysis, there is a significant reduction in the biomass of PP and PET (paired t-test, $t(2) = 8.0$, $p < 0.05$).

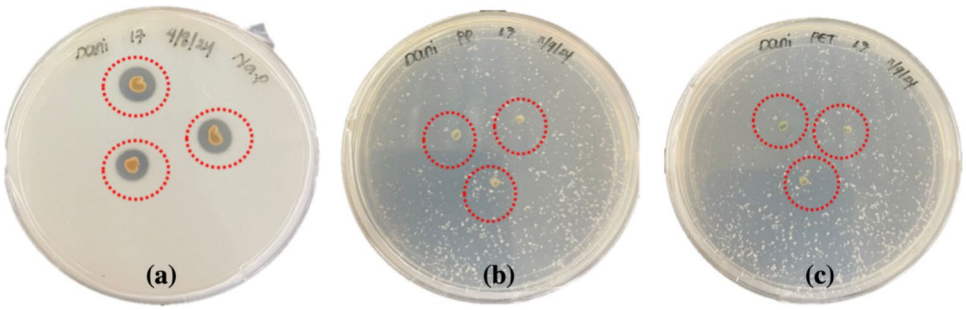

Fig. 1 Phosphorus solubilisation potential and growth response of *Chroococcus* sp. on various insoluble phosphorus substrates. (**a**) Distinct halo zones formed around *Chroococcus* sp. colonies on NBRIP agar amended with sodium phytate as the sole insoluble phosphorus source, indicating active phosphorus solubilisation. (**b**) Visible growth of *Chroococcus* sp. on NBRIP medium supplemented with polypropylene (PP) plastic as its sole phosphorus source, after seven days of incubation. (**c**) Growth of *Chroococcus* sp. on NBRIP medium containing polyethylene terephthalate (PET) plastic as its sole phosphorus source after seven days of incubation

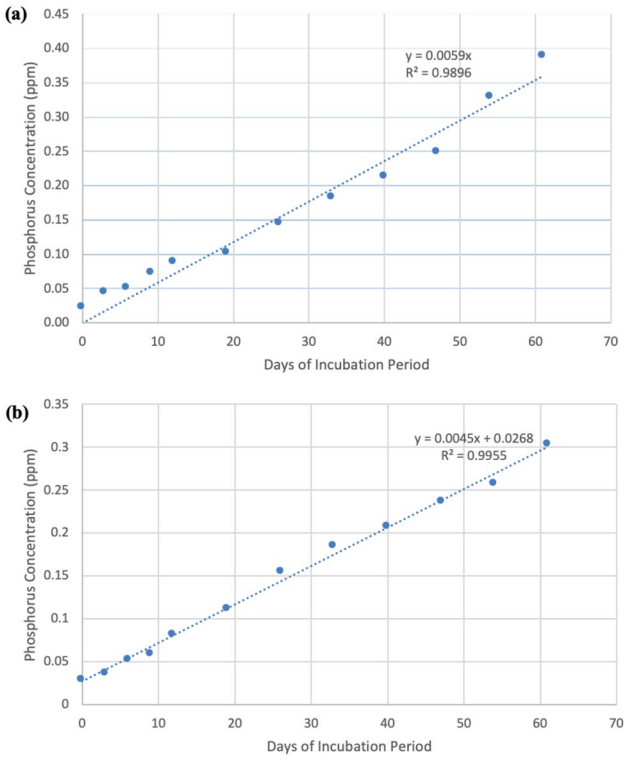

Fig. 2 The amount of soluble phosphorus in the NBRIP liquid medium was determined by measuring the absorbance at 880 nm. (**a**) PP plastic-containing medium both before and after *Chroococcus* sp. incubation. (**b**) PET plastic-containing medium both before and after *Chroococcus* sp. incubation. The potential phosphorus release linked to microbial activity is indicated by the increase in concentration of phosphorus in the NBRIP medium with the incubation period

To complement mass-based observations, scanning electron microscopy (SEM) analysis was performed at magnifications of 800× and 6000× (Fig. 4), revealing surface roughness and localized erosion patterns consistent with microbial interaction, despite the absence of substantial mass loss.

Microbial influence on plastic tensile strength

Tensile strength tests were carried out on the untreated and microbially exposed plastic strips to determine the degree of degradation after incubation for 61 days with phosphorus-solubilising microorganisms. The tensile strength of both PP and PET before and after incubation was compared statistically using a paired t-test. In the case of PP, the mean tensile strength reduced from 76.248 ± 0.215 MPa before incubation to 35.936 ± 0.142 MPa after incubation (mean ± SD; n = 3), with a mean difference between the two values of 40.312 MPa. In the case of PET, there was a drop in tensile strength value from 87.786 ± 0.198 MPa to 45.350 ± 0.177 MPa, with a mean difference of 42.436 MPa. The results of statistical analysis indicate that the reductions in both cases are very highly significant (t = 956; PP t = 3496; $p \ll 0.001$).

Discussion

Molecular identification of cyanobacteria

The commonly accepted criteria for 16S rRNA gene similarity are ≥ 97% for species and ≥ 95% for genus-level classification; hence, this proportion is deemed insufficient for accurate species-level identification. The isolate, tentatively assigned to *Chroococcus* sp., may not belong to any currently recognised or deposited species in the NCBI database, according to a similarity of 93.06%. Rather, it probably refers to a new or uncultured taxon that has not yet been properly described. This finding suggests that a previously unidentified cyanobacterium strain may have been discovered, underscoring the need for additional phylogenetic and morphological research to determine its taxonomic position. No attempt to identify the *phoD*-negative strains was made.

Phosphorus solubilisation from sodium phytate and plastic polymers (PP and PET)

Several metabolic pathways could account for the ability of *Chroococcus* sp. to solubilise phosphorus from both organic and synthetic sources. In the case of sodium phytate, for example, the solubilisation of phosphorus would involve the release of extracellular enzymes such as phytases and phosphatases that hydrolyse the substrate, releasing inorganic phosphate (Pi) in the surrounding media (Jacquin et al. 2019). The halo zones are the result of the breakdown of calcium phosphate due to enzymatic activity.

In addition to enzymatic hydrolysis, microorganisms have been shown to produce organic acids (e.g., gluconic, citric, and oxalic) that decrease the pH of the medium and favour the dissolution of phosphorus-containing compounds, which then release soluble phosphate ions, such as $H_2PO_4^-$ and HPO_4^{2-}, into the environment (Jacquin et al. 2019). For example, this release may be explained by the following chemical reaction: $Ca_3(PO_4)_2 + 2H^+ \rightarrow 3Ca^{2+} + 2HPO_4^{2-}$. When PP or PET plastics were used as phosphorus sources, halo formation was observed, indicating that the cyanobacterium may also take up phosphorus-based additives incorporated into these synthetic polymers. This ability may be enhanced by biofilm formation, in which microorganisms secrete extracellular polymeric substances that allow the organisms to attach to plastic surfaces and may serve as a matrix for extracellular enzymes and other compounds to encounter the plastic substrate and initiate the phosphorus release process and subsequent plastic degradation (Oberbeckmann and Labrenz 2020). This action ultimately

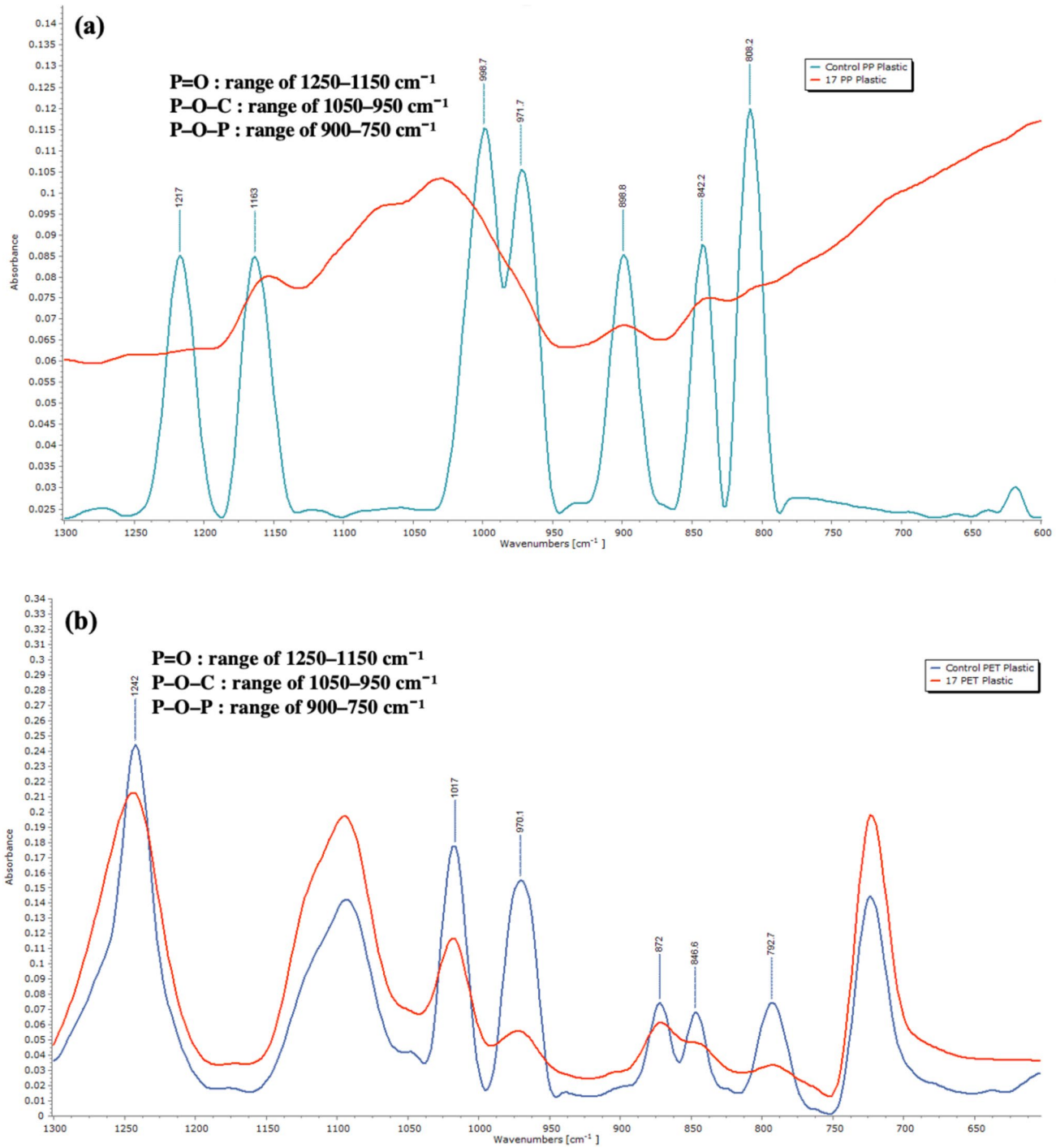

Fig. 3 FTIR spectrum analysis shows the relative strength of phosphorus-related bands in plastic samples. (**a**) PP plastic, before and after a 61-day incubation with the selected cyanobacterium strain. (**b**) PET plastic, before and after the same incubation period. Noticeable band intensity reductions would indicate reductions in phosphorus levels, presumably due to scavenging by the microbes during the process

degrades the plastic surface, promoting further microbial colonisation and enhancing the entire biodegradation process.

Esterases, lipases, and oxidoreductases are some of the enzymes produced by certain bacteria that can interact with plastic matrices to release embedded phosphorus compounds (Lear et al. 2021). The breakdown process can involve oxidative destruction by ROS, which would further increase the release of phosphorus. Furthermore,

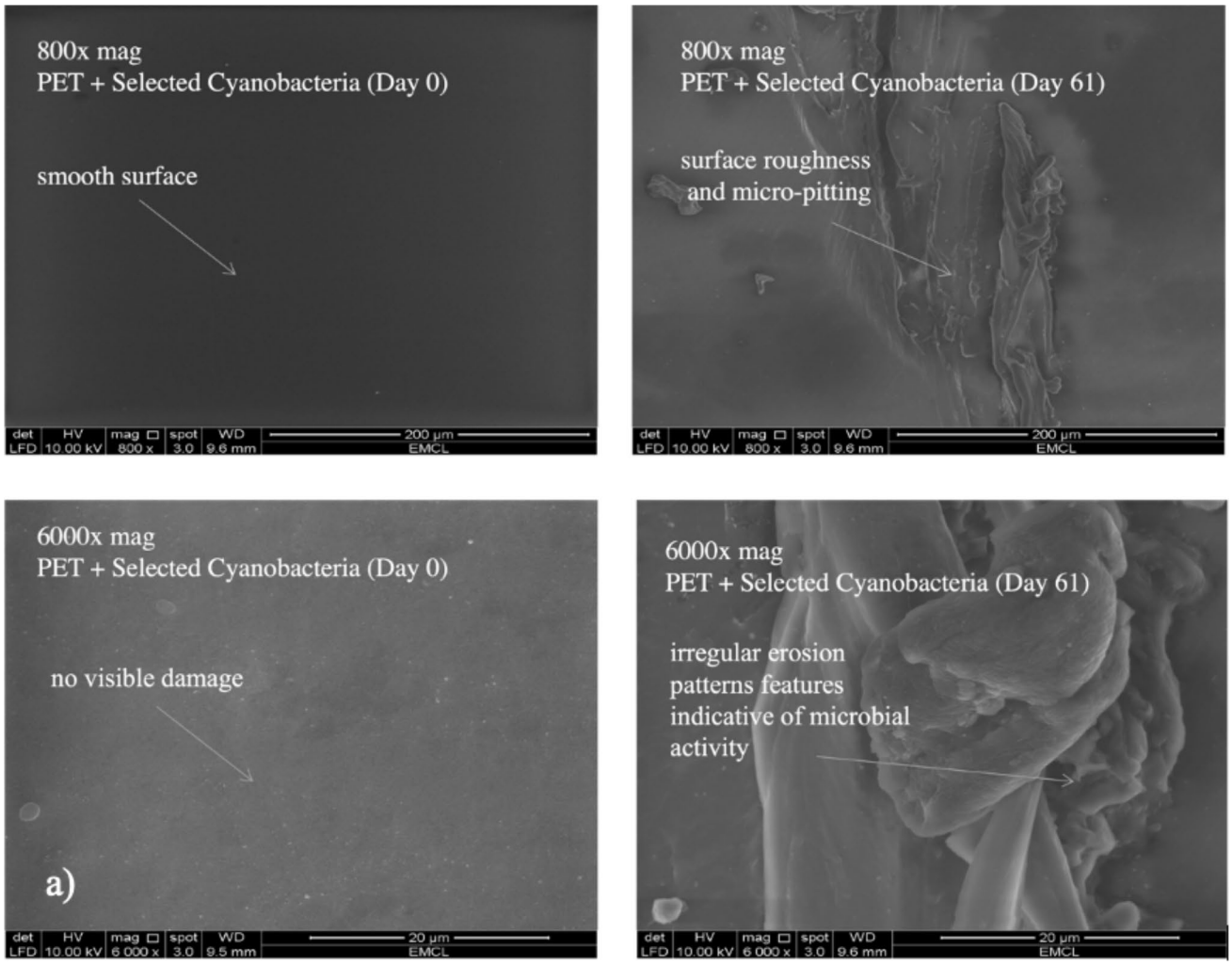

Fig. 4 SEM images showing the surface morphology of (**a**) PET and (**b**) PP plastics at 800× (left) and 6000× (right) magnification, before and after incubation with the selected cyanobacterium. After incubation, both plastics showed evident changes, including increased roughness, pitting, and signs of microbial adhesion, indicating initial phases of surface deterioration due to microbial attack

metabolism with other carbon sources can inadvertently contribute to the release of phosphorus by promoting the degradation of the polymeric components (Wei and Zimmermann 2017). A cyanobacterium capable of thriving on minimum media with PP or PET implies that the strain has developed effective ways of gaining access to phosphorus in nutrient-poor environments (Santos-Beneit 2015). We postulate that such microorganisms can absorb even the minutest amounts of phosphorus because it is distributed through high-affinity phosphate transport mechanisms.

These results suggest that this cyanobacterium strain might utilise phosphorus from organic and synthetic sources through the combined effects of enzymatic activity, acid production, surface attachment, and oxidative reactions. They are capable of utilising phosphorus derived from plastic and, therefore, form a potential candidate for applications in nutrient cycling as well as plastic bioremediation. This would enhance the availability of phosphorus in natural ecosystems, besides providing a novel approach to dealing with plastic pollution. Based on these observations with NBRIP agar plates, we proceeded to quantify the potential of the cyanobacterium strain to scavenge phosphorus from plastic material under liquid culture conditions.

Phosphorus mobilisation from PP and PET by cyanobacterial liquid medium

Although there is considerable experimental heterogeneity, the combined examination of the PP and PET graph figures shows consistent phosphorus (P) solubilisation trends by *Chroococcus* sp. Phosphorus concentration in the medium and the length of PP plastic incubation have a significant

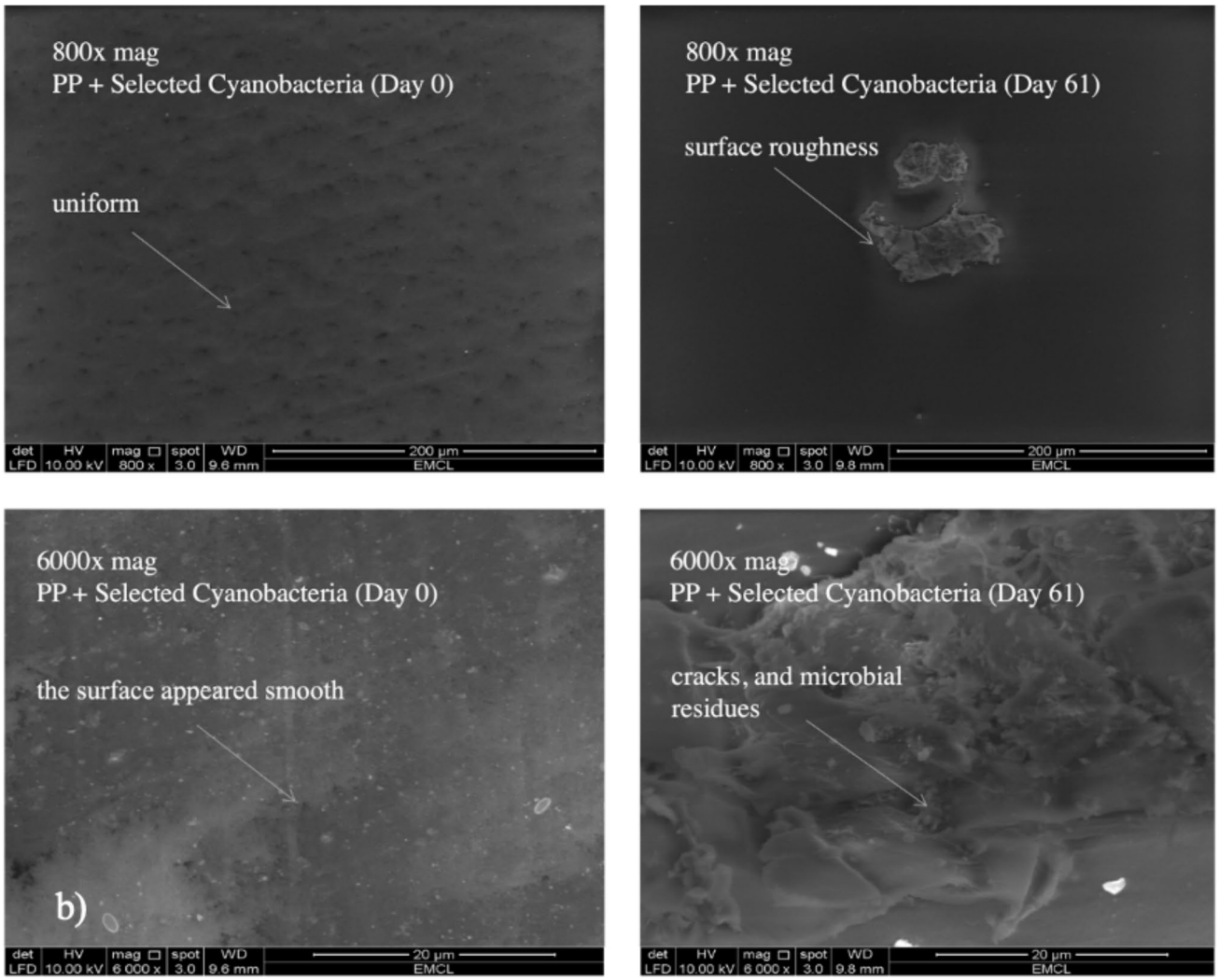

Fig. 4 (continued)

linear connection ($y = 0.0059x$, $R^2 = 0.99$) in Fig. 2a, suggesting rapid P release over time, perhaps due to vigorous cyanobacterium development and organic acid production. For PET plastic, Fig. 2b displays a similar but less noticeable trend ($y = 0.0045x + 0.0268$, $R^2 = 0.99$), suggesting slower phosphorus mobilisation. The initial biofilm formation or background P could be indicated by the increased y-intercept. The variability highlights the need for standardised techniques in light exposure, plastic surface area, and microbial density, even though both tests demonstrate that *Chroococcus* sp. can solubilise P from plastics. These results demonstrate the strain's potential for P recovery and plastic bioremediation; nevertheless, in order to fully comprehend the underlying biological mechanisms, more research needs to examine non-linear growth phases.

The results of this investigation offer compelling proof that, in phosphorus-limited environments, the chosen cyanobacterium strain can mobilise phosphorus from both polypropylene (PP) and polyethylene terephthalate (PET) plastics. The strain can successfully access and release phosphorus bound within or on the surface of various polymeric materials, as evidenced by the steady rise in soluble phosphorus content as determined by the molybdenum blue assay.

FTIR-based analysis of phosphorus content by comparing band magnitude change

Different colours were used to clearly mark the spectra for each condition. For example, the PP/PET control samples before incubation are coloured blue. The samples treated with *Chroococcus* sp. after microbial incubation are coloured orange. Such colour coding makes it easier to identify changes in the spectra associated with phosphorus solubilisation. Microbial activity that degrades or breaks the phosphorus-containing bonds within the plastic matrix is associated with the band intensity decrease, especially in

the 1250–1150 cm^{-1} range corresponding to P = O stretching, the 1050–950 cm^{-1} range related to P–O–C ester linkages, and the 900–750 cm^{-1} range, which corresponds to P–O–P asymmetric stretching. This result suggests that active access and utilisation of the phosphorus molecules, which were either adsorbed onto the surface or within the polymer structure of the plastic material, were taking place through the action of the phosphorus-solubilising microbes and/or the cyanobacterium strains concerned, particularly those verified to harbour the *phoD* gene.

It is possible that the chosen microbial strain reduced the phosphorus content of the two forms of plastic through a variety of connected processes rather than just one. As previously mentioned, the cyanobacterium strain actively eliminates phosphorus by natural absorption for cellular growth and metabolic processes, demonstrating its efficiency in nutrient digestion. Furthermore, localised microenvironments on the plastic surface can be improved by biofilm growth and surface colonisation, encouraging enzymatic activities (such as alkaline phosphatases) that release bound or adsorbed phosphorus compounds. Because of differences in surface chemistry and structure, the PP and PET polymers showed different patterns of phosphorus content reduction. For example, compared to the more hydrophobic and chemically inert PP, the ester functional groups in PET may promote better microbial adhesion or hydrolytic interactions with phosphorus-containing substances. These variations suggest that the observed phosphorus reduction was caused by both biological absorption and plastic–microbe surface interactions.

The different chemical and surface properties of PET and PP blends can be responsible for the difference in the degree of absorbance band magnitude decrease. The polar ester functional groups found in PET may have contributed to increased microbial colonisation, enzymatic activity, and adsorption of phosphorus-containing substances. Stronger connections between microbial phosphatases and phosphorus substrates may have been made possible by increased polarity, which would have improved solubilisation. On the other hand, because PP is non-polar and chemically inert, microbial adhesion and access to phosphorus compounds adsorbed on or embedded within the polymer matrix may be limited.

These results strengthen the hypothesis that microbes may play a role in plastic bioremediation by helping release phosphorus and potentially accelerating plastic breakdown in polluted environments.

Microbial effects on the physical properties of PP and PET

The small but statistically significant differences in biomass for both PP and PET show some degree of material degradation during the experiment period. The material loss can be explained by the presence of surface biodegradation or leaching due to microorganism action and/or physicochemical changes in the incubation medium. The close level of biomass loss for both PP and PET could suggest that the preliminary degradation is related to only surface degradation, which is less likely considering the recalcitrance of the used materials and the short incubation period. However, the statistically significant differences found provide a clear indication of the materials' sensitivity to degradation.

Clear changes in the surface structure of both PP and PET plastics were observed using SEM. The untreated PP surface was smooth and uniform, with no visible defects or damage. In contrast, post-incubation images showed irregular erosion patterns, micro-pitting, and increased surface roughness, indicating microbial interaction. These features suggest localized polymer disruption likely associated with metabolic or enzymatic activity of the cyanobacterium, potentially involving oxidative enzymes or exopolysaccharides that initiate surface modification.

Similarly, the untreated PET surface appeared smooth and intact, without pronounced features. However, after incubation, the PET samples exhibited distinct deformities, including wrinkle lines, fissures, and lumpy or nodular textures. While some of these structures may be attributed to microbial attachment or biofilm formation, the presence of physical damage such as cracks and surface irregularities indicates that the observed changes extend beyond simple surface colonisation.

Taken together, these observations suggest that P-solubilising cyanobacteria not only attach to plastic surfaces but also contribute to early-stage degradation through surface-level interactions, supporting their potential role in nutrient cycling and microplastic breakdown.

Impact of microbial activity on plastic tensile strength

While the changes in the biomass were relatively small, both PP and PET showed notable declines in their tensile strengths after exposure, implying significant damage to their mechanical properties. It appears likely that there is some kind of degradation in the molecular structure of the polymers, such as the breakage of molecular chains or cracks at the molecular level, before there is any considerable change in the mass of the materials. The difference between the tensile strengths of PP and PET may lie in their structures, particularly the fact that PET contains an ester linkage that is prone to hydrolysis.

These large, relatively uniform declines in tensile strength over all replicates indicate severe mechanical property degradation for both polymers during prolonged ageing due to microbial attack. This reproducibility implies that such changes in results are due to real

microbial-induced degradation, not random measurement error. The magnitude and consistency of tensile loss emphasise the important role that phosphorus-solubilising microorganisms play in reducing both surface and structural strength.

Conclusions

The present study degrades PET and PP plastics that contain phosphorus additives by cyanobacteria. Decreases in plastic mass, tensile strength, and structural integrity were confirmed by physical, chemical, and molecular analyses to result from phosphorus solubilisation by favouring microbial colonisation. These results emphasise the potential contribution of phosphorus solubilisation to integrated waste management methodology as a bioremediation tool. It is suggested that information in the future should be reinforced through clarification of the microbial mechanism, optimisation of physiological environmental conditions, and evaluation of scalability regarding practical applications.

Environmental Implication

The presented research indicates that phosphorus-solubilising cyanobacteria weaken polypropylene (PP) and polyethylene terephthalate (PET) through the extraction of phosphorus-based additives, reducing their structural integrity and showing initial surface degradation. This nutrient-driven mechanism offers a novel route to plastic breakdown independent of carbon assimilation, which may accelerate fragmentation in natural environments. These microbial processes can increase weathering rates of plastic waste, enhance nutrient cycling, and support integrated bioremediation strategies for persistent plastics in aquatic and terrestrial ecosystems.

Appendix: Sequence of *Chroococcus* sp. 16S sRNA gene

GTGTAATCGCAGCCGTATCCGCTCATCCTGTAGCTA
GGATACACACTCATCCAACTACTTCGGGCGTGCCGC
TCCCATGGGTGTGACGGGCGGTGTGTACAAGGC
CCGGGAACGTATTCCCCGCAGTATGCTGACCTGCGA
TTACTACCCAATCCGCCTTCATGCACGCGAGTTGCA
GCCTGCTATCTGAACTGTCCCCCGCTAAATCTCTAT
TGCGACGTTCCCCTGGCCGACCCCCTGTGGCTTTAT
CACACTCTCACATTGCGGCGGGAGA

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Author contribution J.L. conceptualized and designed the study and provided primary supervision. D.W. led data acquisition and prepared the first draft of the manuscript, including initial analysis. J.L., S.A.M., and P.C. contributed to editing and finalizing the manuscript. J.A.R. and L.H.C. supported the acquisition of the data. A.K. and N.H. critically reviewed the manuscript and gave important feedback. All authors reviewed and approved the final version of the manuscript.

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Data availability Data validating the results obtained from this study are publicly accessible through the data repository of Universiti Sains Malaysia, with the title 'Biodegradation of Plastics by Phosphorus-solubilizing Cyanobacterium Chroococcus sp.' and are available at the following URL: https://tidbrepo.usm.my/database/2ff28baa-f6da-455d-aaed-a7553a7dab9f/info?pid=f16ed1a0-184c-43a3-bb10-45e49e6ffbcf

Declarations

Conflicts of interest The authors declare no competing interests.

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