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Research Paper

Biodegradation of bisphenol-A polycarbonate plastic by *Pseudoxanthomonas* sp. strain NyZ600

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ABSTRACT

Bisphenol-A polycarbonate (PC) is a widely used engineering thermoplastic and its release has caused damage to the ecosystem. Microbial degradation of plastic represents a sustainable approach for PC reduction. In this study, a bacterial strain designated *Pseudoxanthomonas* sp. strain NyZ600 capable of degrading PC was isolated from activated sludge by using diphenyl carbonate as a surrogate substrate. Within a 30-day period of incubating with strain NyZ600, PC films were analyzed with atomic force microscopy, scanning electron microscope, water contact angle, X-ray photoelectron spectroscopy, fourier transform infrared spectroscopy, differential scan calorimeter and thermogravimetric analysis technique. The analyses results indicated that the treated PC films were bio-deteriorated and formed some "corrosion pits" on the PC film surface. In addition, strain NyZ600 performed broad depolymerization of PC indicated by the reduction of Mn from 23.55 to 16.75 kDa and Mw from 45.67 to 31.97 kDa and two degradation products bisphenol A and 4-cumylphenol (the two monomers of PC) were also found, which established that PC were biodegraded by strain NyZ600. Combing all above results, it is clear that the strain NyZ600 can degrade PC which provides a unique example for bacterial degradation of PC and a feasibility for the removal of PC waste.

1. Introduction

Since the global production of synthetic plastics has exceeded 368 million tons in 2019 (PlasticsEurope, 2020), the release of their wastes to the environment has become a global environmental concern. To remove plastic contaminants in biochemical ways has always been one of the desirable solutions. However, due to the absence or low activity of robust decomposing enzymes that can naturally break down plastic waste, these plastics have significant persistence in the environment (Yoshida et al., 2016). Bisphenol-A polycarbonate (PC) was commercialized by General Electric and Bayer in the 1950s (Kim, 2020) and this polymer is formed by the condensation reaction of a carbonyl source (generally phosgene or diphenyl carbonate) with bisphenol A (BPA) (Brunelle et al., 2005). This engineering thermoplastic is widely used in various industrial fields, such as building and construction, automotive and transportation, optical and ophthalmic media, medical, packaging and optical data storage devices (Siddiqui et al., 2018). In 2017, PC production has reached a total of 4 million tons (Zhang et al., 2019) and its production is predicted to gradually increase to 5 million tons by the end of 2023 (Kim, 2020). One of the ways that PC wastes can bring harm to environment and human health is that PC microplastics were also detected in dust (Liu et al., 2019) and sewage sludge samples (Zhang et al., 2019). Additionally, it was reported that the intake of PC microplastic via dust for infants and adults was 7.37 ng/kg-bw/day and 0.5 ng/kg-bw/day respectively during the detection period of three months in 39 urban cities of China (Liu et al., 2019). Hence, the release of PC may likely cause damage to the environmental ecosystem and human health. Consequently, the increased generation of plastic wastes and

List of abbreviations: PC, Bisphenol-A polycarbonate; AFM, atomic force microscopy; BPA, bisphenol A; FTIR, fourier transform infrared; SEM, scanning electron microscope; TGA, thermogravimetric analysis; DSC, differential scan calorimeter; GPC, gel permeation chromatography; WCA, water contact angle; XPS, X-ray photoelectron spectroscopy; GC-MS, gas chromatography-mass spectrometry; PET, polyethylene terephthalate; LEM, Liquid enrichment media; LCFBM, liquid carbon free basal medium; MWD, molecular weight distribution; Mw, weight-average molecular weight; Mn, number-average molecular weight; Mz, size-average molecular weight; Tg, glass transition.

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their the subsequent elimination have become a growing concern over the past few decades. Several chemical and physical means have been demonstrated to enhance the degradation rate of PC (Kim, 2020; Siddiqui et al., 2018; Sivalingam and Madras, 2004a). In the chemical treatment for PC degradation, the use of toxic organic solvents not only requires a complex product separation scheme but also involves a range of environmental and safety issues (Antonakou and Achilias, 2013). On the other hand, the photo and thermal degradation provide safer options, but many unwanted by-products are produced in the degradation processes (Antonakou and Achilias, 2013; Rivaton et al., 2002). Apart from chemical and physical treatment for PC degradation, microbial degradation has attracted scientists' attention. Lately, microbial treatment is considered to be used as an environment friendly alternative to degrade plastics. An *Arthrobacter* strain isolated from polyethylene contaminated waste disposal sites was thought to be able to degrade PC plastic, evidenced simply by fourier transform infrared (FTIR) spectra, which proved that polycarbonate was converted into a polar by-product (Goel et al., 2008). More recently, a study on bacterial degradation of polycarbonate plastic with undescribed chemical structure, in which surface roughness changes of the polycarbonate film were detected and a peak of new product was characterized by atomic force microscopy (AFM) and FTIR respectively (Arefian et al., 2020). These results suggested promising signs of PC biodegradation by bacteria but genuine biodegradation has not been confirmed with hard evidences. In addition, biodegradation studies with several fungi were also reported. *Geotrichum candidum* can eat compact disk (CD) by producing spores which bore holes in CD, rendering them useless (Bosch, 2001). A white rot fungus from a culture collection was found to be able to cause 5.4% weight loss for UV-treated PC after one year treatement (Artham and Doble, 2010). Five commercial lipases from fungi and porcine pancreas, as well as two commercial lipases of Lipolase and Novozyme, were also found to be able to catalyze PC degradation in organic solvents (Artham et al., 2011; Sivalingam and Madras, 2004b). It was also reported when *Amycolatopsis* sp. strain HT-6 was inoculated into a liquid medium with 150 mg poly (tetramethylene carbonate) film, the weight loss of this film reached 59% (Pranamuda et al., 1999). Compared with aliphatic poly (tetramethylene carbonate), aromatic PC is more difficult to be degraded by microorganisms. Nevertheless, as an important domain in life science and with diverse catabolic potentials, bacterial PC degradation methodology has not been well studied with more detailed and sufficient evidences to show their capabilities of PC degradation.

Here, a two-step bacterial strain screening scheme was established for effective isolation of strains capable of degrading PC from environmental samples. PC degrading bacterial strain NyZ600 was isolated by using diphenyl carbonate as a surrogate substrate, which has carbonate bonds similar to the repeat unit of PC. The chemical and physical properties of degradation products as well as the surface of the treated film were characterized with a variety of means including AFM, FTIR, scanning electron microscope (SEM), thermogravimetric analysis (TGA), differential scan calorimeter (DSC), gel permeation chromatography (GPC), water contact angle (WCA), X-ray photoelectron spectroscopy (XPS), and gas chromatography-mass spectrometry (GC-MS). The results from these assays convincingly established that the strain NyZ600 can degrade PC, providing a unique example for bacterial degradation of PC and a feasibility for the removal of PC waste. Strain NyZ600 can transform PC into catabolites bisphenol A and 4-cumyl phenol, indicating an exo-type (end-chain scission) degradation mechanism adopted in the degradation, which has never been reported for bacterial PC degradation. Thus, different degradation pathways may have occurred in PC degradation between strain NyZ600 and fungi. In summary, given the fact that very limited research studies have been published on PC microbial degradation, current discovery of strain NyZ600 for PC degradation has certainly expanded the resource library of microbial degradation of PC, making a significant contribution to plastic degradation, especially for PC.

2. Materials and methods

2.1. Materials

PC pellets [weight-average molecular weight (Mw): 45.67 ± 1.39 kDa, number-average molecular weight (Mn): 23.55 ± 0.618 kDa and size-average molecular weight (Mz): 73.44 ± 2.762 kDa] were purchased from Sigma-Aldrich (CAS No: 25037-45-0). To prepare PC film for bacterial degradation, PC pellets (2 g) were dissolved in 100 ml of dichloromethane (HPLC grade). This solution (5 ml) was dropped on a glass Petri dish (Φ 90 mm). The solvent was allowed to evaporate and a film was formed. The PC films were removed and placed in a fume hood at room temperature for 2 days to thoroughly evaporate the dichloromethane. The PC films were sterilized with 75% ethanol (*v/v*) and rinsed with sterile water. They were then cut into sheets of 50×50 mm and 30 × 30 mm square for bacterial colonization on agar plates and for the growth of bacterial suspension in a liquid medium respectively.

2.2. Enrichment and isolation of PC-degrading bacteria

The activated sludge samples were derived from the aeration stage of the municipal wastewater treatment plant in Suzhou, China. Approximately 3 g of the samples were cultivated in an Erlenmeyer flask containing 100 ml of liquid enrichment media with PC film (ca. 700 mg, 30 × 30 mm). Liquid enrichment media (LEM) per litre consists of: 1.5 g K_2HPO_4, 0.5 g KH_2PO_4, 1.0 g NH_4NO_3, 1.0 g NaCl, 0.2 g $MgSO_4$, 0.01 g glucose, 0.01 g peptone, 0.002 g $FeSO_4{\cdot}7H_2O$, 0.002 g $ZnSO_4{\cdot}7H_2O$, 0.001 g $MnSO_4{\cdot}H_2O$, 1% (vol/vol) glycerol tributyrate and the pH of the media was about 7.0–7.2. The Erlenmeyer flask was maintained in a shaking incubator at 180 rpm and 30 °C.

Residual PC pieces with solutions were removed to fresh liquid enrichment media after 10 days incubation. After three cycles of enrichment, the solution was coated onto LB (lysogeny broth) agar plates containing 1% (vol/vol) glycerol tributyrate (pre-emulsified with 1.2 g liter^{-1} polyvinyl alcohol using a vortex) and 15 g liter^{-1} agar. The plates were incubated at 30 °C until clear zones (halo) were detected around single colonies (≤7 days). Positive bacterial colonies were further evaluated for their hydrolysis activity of carbonate bonds using diphenyl carbonate as a substrate. For positive colonies with activity, the carbonate bond in diphenyl carbonate will be hydrolyzed to form phenol, which will bind to ferric trichloride to form a visible purple complex (Wesp and Brode, 1934). The positive strains were further tested for their production of PC catabolites in liquid media. A bacterial strain designated NyZ600 was finally isolated in this way. The 16S rRNA gene was amplified by PCR using universal primers 27F and 1492R (Lane, 1991).

2.3. Evaluation of the capabilities of strain NyZ600 for degrading PC film

Strain NyZ600 was cultured in liquid LB medium containing 1% (vol/vol) glycerol tributyrate overnight to an optical density at 600 nm (OD_{600}) of 0.8 and then washed twice with liquid carbon free basal medium (LCFBM, which is LEM without glycerol tributyrate, glucose and peptone) and then resuspended in equal volume of LCFBM. Strain NyZ600 was inoculated in 100 ml LCFBM with 1 g PC film on a rotary shaker (180 rpm) at 30 °C. After a 30-day incubation, the PC films were washed adequately with 2% SDS and then sterile water before being dried for assay. In addition, PC films without bacteria inoculation served as negative controls.

2.4. Topographical, physical and chemical characterizations

AFM technique was employed to intuitively observe the deterioration of plastic film surface by bacteria. The morphology of PC film surfaces was also characterized by SEM measurements (HITACHI, S3400II, Japan). The detailed methods of AFM and SEM were introduced in the

Text.S1 of Supporting Information.

The water contact angles were measured via an optical video contact angle instrument (DSA100, Kruss, Germany) at room temperature. 5 μl of deionized water was dropped onto the surface of PC film and the contact angles were obtained from 4 different contact positions on each PC film. The surface chemical compositions of the PC films were analyzed by XPS technique. The XPS measurement was carried out on a Kratos Axis Ultra DLD spectrometer (Shimadzu-Kratos, Japan) using a monochromatic Al Ka source (7 mA, 15 Kv) with the energy of 1486.6 eV. The photoelectrons were detected at a take-off angle of 90°. Survey spectra and high resolution spectra were acquired with an analysis area of 300 μm × 700 μm. Pass energies of 160 eV and 40 eV were used for survey scan and high resolution scan, respectively. The binding energy scale was calibrated according to the C 1s peak (284.8 eV) of adventitious carbon on the sample surface. The curve fitting for the high resolution spectra were conducted using Casa XPS 2.3.23 software.

2.5. Characterization of molecular weight distribution

For GPC analysis, the PC films (10 mg) were dissolved in 10 ml of tetrahydrofuran. The solution was filtered through a 0.22 μm syringe filter prior to the injection. The molecular weight distribution of PC films was analyzed by GPC using HLC-8320GPC (Tosoh Corp, Japan). The GPC instrument contains an RI detector (RI testing conditions: Pol(+), Res(0.5 s)), a chromatographic column (TSK gel Super Multi pore HZ-M (21488)), and a guard column (TSK guard column Super Multi pore HZ-M (21489)). The chromatographic column was used to separate the molecules using tetrahydrofuran as an eluent. After balancing the column, the flow rate was set to 0.35 ml/min at 40 °C. The filtered solutions (20 μl) were then injected onto the column for analysis. Standard calibration was performed with Mw standards of polystyrene (266 Da, 370 Da, 474 Da, 5.97 kDa, 96.4 kDa, and 706 kDa) from PS Quick MP-M, Tosoh Corp.

2.6. Observation of PC film degradation

Samples were dried in a 50 °C oven for at least 15 h prior to the thermal investigation. The PC samples were analyzed by thermogravimetric analyzer (Pyris-1 TGA, PerkinElmer) with temperature ranging from 30 °C to 700 °C. Approximately 4 mg of samples were used for TGA and all experiments were carried out under nitrogen atmosphere, with a flow rate of 100 ml/min and linear heating rate of 10 °C/min.

To estimate the glass transition temperature of PC samples, the NETZSCH Phoenix DSC (NETZSCH 204 F1) was calibrated for temperature and enthalpy measurements. Approximately 2 mg of each sample was loaded into an aluminum pan and placed at on an appropriate position in the DSC instrument. DSC was performed under nitrogen atmosphere at a scanning rate of 10 °C/min. The DSC experiments were carried out as follows: the samples were heated from 20 °C to 300 °C and then cooled down to 20 °C before being heated to 300 °C again. To eliminate heat history, this procedure was done twice and the thermogram of the second scan was used for the determination of glass transition temperature. The glass transition temperature (Tg) was estimated using the mid-Cp interpolation method (Tsintzou et al., 2012). FTIR (Nicolet 6700, Thermo Fisher Scientific, U.S.A.) was used to detect the formation of new functional groups in the frequency range of 4000–500 cm^{-1}. The analysis was performed using attenuated total reflectance (ATR) mode by accumulating 32 scans (Artham and Doble, 2010).

2.7. Characterization of catabolites by GC–MS

PC film (1 g) was incubated with the bacterial cells in LCFBM for 5 days and possible catabolites from PC film degradation were extracted twice from the culture with equal volume of dichloromethane. The resulting organic phases were decanted and pooled, dehydrated with anhydrous Na_2SO_4, and further concentrated by revolving evaporation. Next, the catabolites were dissolved with pure dichloromethane and subjected to 0.22 μm syringe nylon membrane filters prior to GC-MS analysis.

PC degradation catabolites analysis was carried out on a GC-MS system (Agilent, USA) consisting of a 7890B-GC with MMI inlet, and 5977B-MS with a quadrupole mass spectrometer. Separation of metabolites were performed on an HP-5MS columns (30 m × 0.25 mm × 0.25 μm). The interface and the source temperature were 290 °C and 230 °C, respectively. Electron impact mass spectra were recorded in the 33–750 *m/z* mass range under 70 eV ionization energy. The oven temperature program was maintained at 70 °C for 2 min and raised to 130 °C at 5 °C/min, increased to 180 °C at a rate of 10 °C/min, then to 285 °C at 5 °C/min, which was held for 1 min.

3. Results and discussion

3.1. Isolation and identification of PC degraders

After three cycles of enrichment, a total of 11 bacterial strains were isolated from the PC enrichment media. Positive colonies would form clear zones on triglyceride plates and were then evaluated for their hydrolysis activity for carbonate bonds using diphenyl carbonate. Formation of a purple complex with ferric trichloride was an indication of presence of hydrolysis activity, as shown in Fig. S3a.

Three bacterial colonies were found to show hydrolysis activities with diphenyl carbonate, forming a distinct purple complex. These three isolates were then cultured on PC films to examine the formation of BPA in liquid media. Among these three isolates, only one strain designated NyZ600 showed a positive result on the formation of BPA. Strain NyZ600 was finally chosen for further analyses by biotransformation of diphenyl carbonate (DPC) in phosphate buffer at a final optical density of 10.0 at OD_{600}, resulting in formation of 10.8 mmol/L phenol as a final product after an hour incubation (Fig. S3b). This strain is a Gram-negative rod and non-spore-forming bacterium. Sequence analysis of its 16S rRNA gene showed that it belongs to *Pseudoxanthomonas* sp. (Fig. S1). The 16S rRNA gene sequence (1415 bp) was deposited in GenBank with Accession No. MT560351.

The strain NyZ600 was inoculated into LCFBM containing PC in 30 days, the cell density increased from an OD600 of 0.02–0.035 ± 0.008, while the cell density in control sample without inoculation increased from an OD600 of 0 to 0.003. These results indicated that strain NyZ600 had virtually no increase in biomass when inoculated in LCFBM with PC as the sole carbon source. Therefore, LB agar plates containing 1% (vol/vol) glycerol tributyrate, covered with PC film (approximately 60 mg), were used to examine the weight loss of PC film after incubation with strain NyZ600. After 30 days incubation, 2.5% weight loss of PC film was detected and prolonged incubation (> 30 days) did not cause a significant increase in this loss (Fig. S2).

Discovery of the PC degrader strain NyZ600 indicates the presence of PC degrader bacterial strains in the activated sludge. It was reported that PC microplastics were detected in activated sludge (Zhang et al., 2019) and bacteria may have been domesticated to be capable of degrading this type of plastics after a long period of enrichment in activated sludge.

In total, three bacterial strains from different genus displayed the abilities to dissociate the carbonate bonds in bicarbonate compound, but only strain NyZ600 breaks the carbonate bonds of PC. This result indicates that plastic polymer may require specific hydrolyzing enzymes with a more suitable substrate binding pocket. During the degradation of PET plastics, the broad substrate binding pocket in PET hydrolase (PETase) is the key for its ability to hydrolyze PET. The surface of PETase has been close to 40 Å of slender groove as the substrate binding cleft (Joo et al., 2018). Moreover, PETase has more open active site cleavage than homologous cutinases (Austin et al., 2018; Liu et al., 2018). From our case in this study, it is understood that enzymes degrading diphenyl carbonate may not necessarily hydrolyze the carbonate bonds of PC. Then screening for a variety of diphenyl carbonate degraders may

provide us more chances to obtain functional PC degraders of diverse origins, or a candidate enzyme from which functional enzymes toward PC can be generated via protein engineering.

3.2. Changes in physical, chemical and topographical properties of plastic film surface after strain NyZ600 treatment

AFM images detection showed the changes in the surface morphology and roughness in situ degradation of the PC films treated with strain NyZ600 after 30 days incubation. Topographic change indicating biodegradation were concluded from evidently higher values for Ra (untreated, 0.236 nm; biotic treatment, 2.194 nm), Rq (untreated, 0.294 nm; biotic treatment, 4.404 nm), Rz (untreated, 2.478 nm; biotic treatment, 65.477 nm), as shown in Fig. 1. It was considered that significant increases in Ra, Rq and Rz indicate the changes in the topography of plastic biodegradation. (particularly, Rz values between large peaks amplitude of biotic and abiotic treatments indicate the formation of wide and deep cavities (Peixoto et al., 2017). On the other hand, observation by SEM is able to distinguish different treatment methods applied in the plastic degradation, based on special morphology characteristics on surface. The SEM analyses in this study, the membrane surface of the negative control groups was smooth without any defects as compared to the treatment groups (Fig. 2a, b). Fig. 2c and d illustrated that the inoculation with strain NyZ600 was able to immobilize the cells on plastic film and corroded its surface to form various holes. The presence of holes and rough surfaces on the film may be due to the secretion of bacterial extracellular enzymes to enable this corrosion, similar to a report where PET plastics were corroded by the cells of a PET degrader (Yoshida et al., 2016). Physical and chemical treatments, in contrast to hole formations in microbial degradation, will usually cause the formation of long cracks on the PC film (Artham and Doble, 2010). The occurrence of roughness and holes of the film surface shown in Fig. 2c and d indicated that the PC film polymer structure was destroyed to some extent, due to biological factors. Collectively, these contributed to the 2.5% weight loss of the plastic film over the course of a 30-day treatment.

The water contact angles were measured to determine changes in surface hydrophobicity of the PC films. According to the value of the

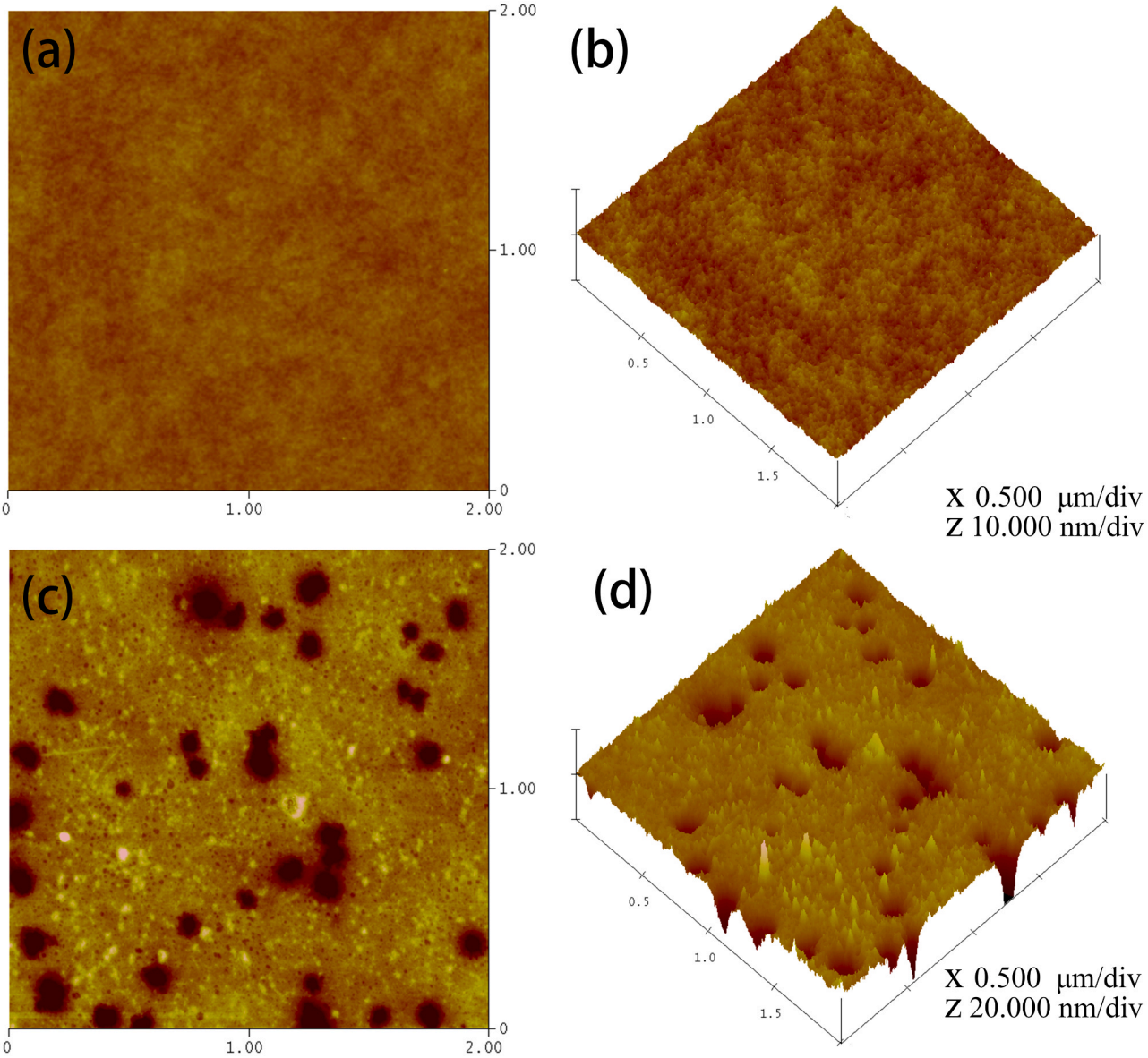

Fig. 1. Two dimensional (2D) and three dimensional (3D) atomic force microscope (AFM) images of the physical surface topography: (a) 2D and (b) 3D images of the control sample; (c) 2D and (d) 3D images of the PC films treated by strain NyZ600 for 30 days.

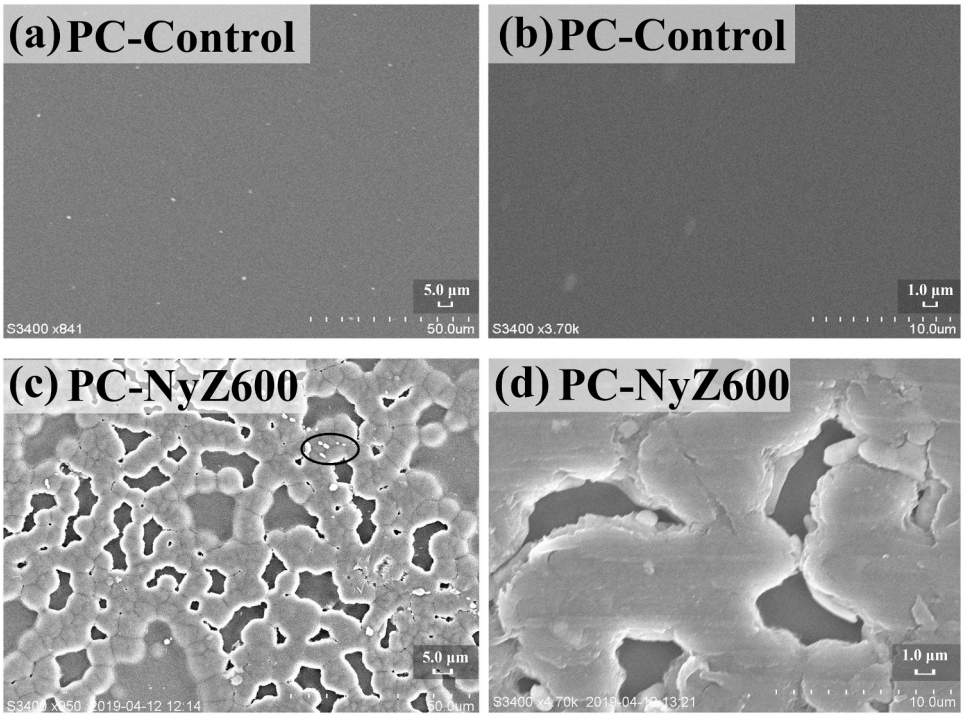

Fig. 2. Scanning electron microscopy (SEM) images of the control sample (a and b) and PC film degradation by strain NyZ600 for 30 days (c and d). An ellipse indicates the presence of bacterial cells (c).

contact angle, the surface properties are determined to be hydrophilic (contact angle $< 90°$) or hydrophobic (contact angle $> 90°$) of the tested polymers (Peters, 2017). The contact angles of the control sample of untreated in this study were measured to be $97.9 \pm 0.5°$ ($n = 4$) (Fig. 3a), which was significantly larger than that of the PC film treated with strain NyZ600 ($74.8 \pm 2.2°$; $n = 4$; $P < 0.05$ by Student's *t*-test) (Fig. 3b). The results indicated that the inoculation of strain NyZ600 decreased the surface hydrophobicity and increased water-surface interaction of the PC film. The roughness and holes formed on the surface of the PC film treated with strain NyZ600 were observed by AFM and SEM, resulting in the increased hydrophilicity of PC film surface. Interestingly, a similar observation has been reported in the biodegraded PS film by *Serratia* sp. WSW (Woo et al., 2020).

XPS was used to characterize the elemental content and chemical composition of the sample surface. As shown in Fig. 3e, the survey spectra showed that the PC film surface was composed of O and C elements and the elemental ratio of O/C at the PC film surface treated by strain NyZ600 increased from 15.6% to 18.8%, compared with the control sample. It can be speculated that the treated sample can introduce additional oxygen-containing groups on the PC film surface. To further explore the variation of oxygen-containing functional groups, the high-resolution XPS spectra of C 1s were further analyzed (Fig. 3c,d). The C 1s spectra can be fitted with five components with the Gaussian/Lorenzian ratio of 30/70. These five components were assigned to different bonding states (284.5 eV, aromatic C-H/C-C; 285.0 eV, aliphatic C-H/C-C; 286.2 eV, C-O/C-OH; 294.0 eV, O$^{=}$C-O; 291.3 eV, 292.5 eV, π-π*), according to the standard binding energy and previous literature (Muir et al., 2006). As expected, the PC film surface treated by strain NyZ600 resulted in a mild decrease in the peak area ratio of the carbon connecting two oxygen atom O$^{=}$C-O over total carbon from 4.68% to 4.39% and a significant increase in the peak area ratio of C-O/C-OH over total carbon from 17.27% to 20.48%, indicating some of O$^{=}$C-O groups at PC surface were decomposed into C-OH group (see Table S1). In conclusion, XPS results suggested that strain NyZ600 was capable of breaking down the carbonate bond of PC film surface with the formation of hydroxyl group at the surface.

3.3. DSC analysis: glass transition temperature (Tg) decrease of the PC film by strain NyZ600 treatment

DSC is a thermal analysis method used to measure the change of physical properties of substances at different temperatures. In this study, the specific heat flow of the PC film by bacterial degradation after 30 days is shown in Fig. 4 for DSC analysis. For the control sample, the thermogram of the first and second scan showed similar curves with almost the same Tg (149 °C and 148.9 °C, respectively) as shown in Fig. 4a, close to the one reported for amorphous bisphenol-based polymers which is approximately 150 °C (Delpech et al., 2002). Meanwhile, no melting peaks were found in the control, consistent with that PC is ductile amorphous polymer as reported (Neki and Geil, 1973; Tsuburaya and Saito, 2004).

In contrast, the DSC curves for the treated sample showed evident differences in the two thermal cycles (Fig. 4b). During the first heating cycle, both endothermic (area below the curve) and exothermic (area above the curve) were observed. In the second heating cycle, however, the endothermic and exothermic peaks of the DSC curve disappeared completely. The results suggested that endothermic and exothermic reactions occurred due to the surface degradation of the plastic film by bacterial cells to possibly form low-polymers and smaller molecules. These formed low-polymers and smaller molecules were likely gasified when the first heating process reached 300 °C so that they disappeared in the second cycle. In comparison to the control group (148.9 °C), the glass transition (Tg) temperature was 141.4 °C, 7.5 °C lower than that of the control group in the second cycle. Interestingly, a similar difference (a 7.3 °C decrease) in Tg was also observed after fungal degradation of PC (Artham and Doble, 2010). Admittedly, a drop of 7.5 °C in Tg between the sample and control in this study was not particularly huge. Nevertheless, a difference of 4 ℃ in Tg can be considered as an indication of the increase in the fluidity of the polymer chain, which might be the result of a decrease in the average molecular weight and plasticization caused by hydrolysis (Tsintzou et al., 2012). Therefore, the DSC curve analysis in this study suggested that the surface erosion of PC films occurred after treatment by the bacterial strain.

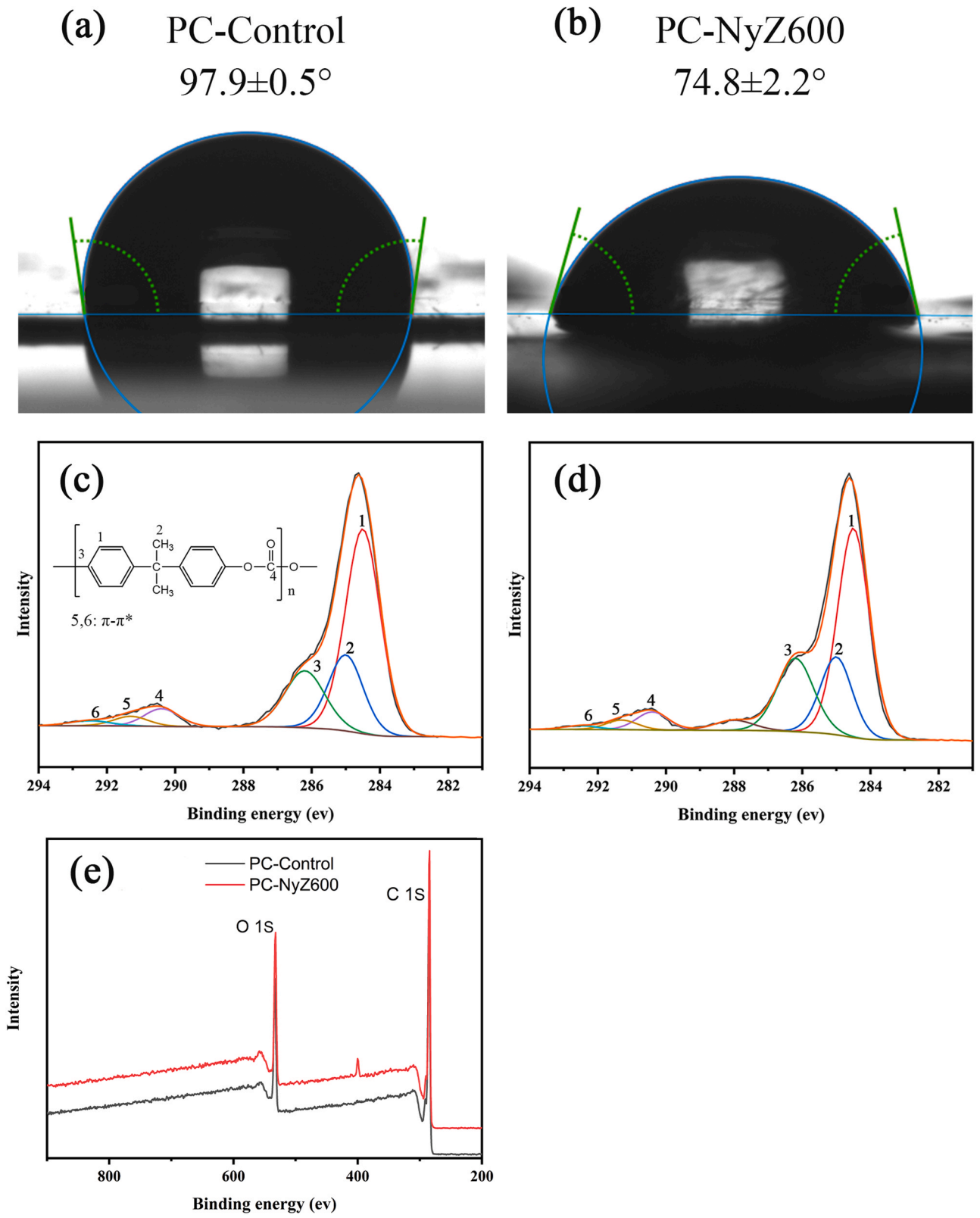

Fig. 3. Surface chemical analysis of the control and the PC films treated by strain NyZ600 for 30 days. (a) Water contact angle (WCA) values of the control PC films (n = 4) and (b) the PC films treated by strain NyZ600 (n = 4), indicating a decrease in surface hydrophobicity. (c, d) The fine-scanned C 1s X-ray photoelectron spectroscopy (XPS) spectra of the control and the PC films treated by strain NyZ600. (e) C1s and O1s XPS full survey spectra of the control and the PC film incubated with strain NyZ600 for 30 days.

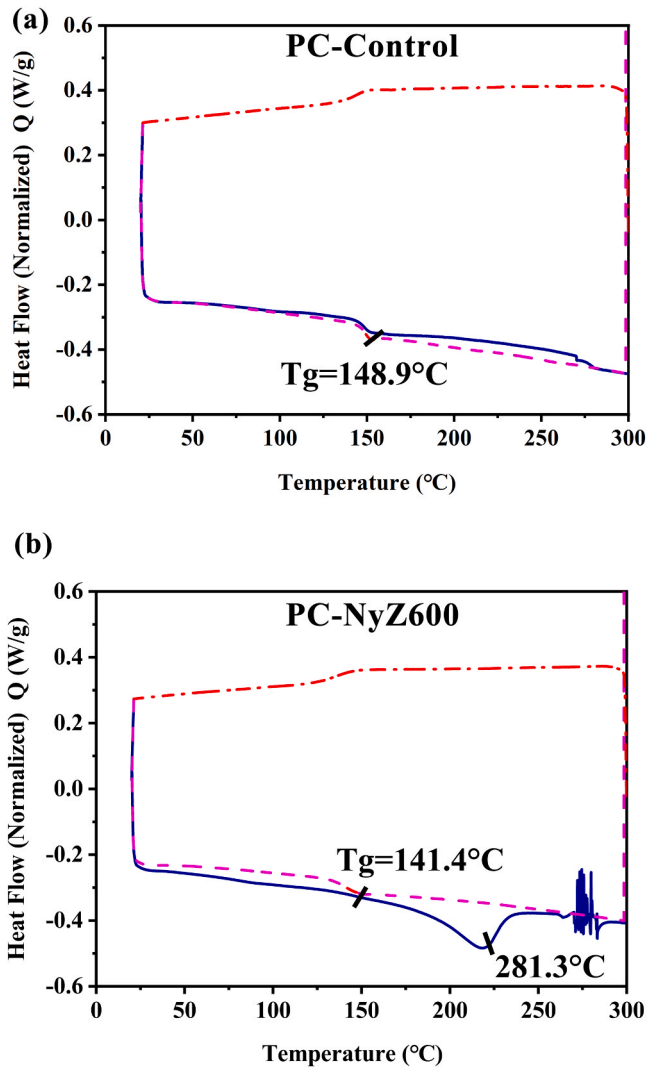

Fig. 4. Differential scanning calorimetry (DSC) curves of heating and cooling cycles of sample. (a) control; (b) treated sample (PC films incubated with strain NyZ600 for 30 days). The solid line represents the first heating process; the dash-dotted line represents the first cooling process; the dashed line represents the second heating process.

3.4. Reduction of thermal stability

TGA is a thermal analytic technique that measures, under programmed temperature control, the relationship between the quality of the sample and the temperature change. It is employed to study the thermal stability and composition of materials including various plastics. The thermal degradation behavior of the plastic samples was often investigated by TGA and differential thermogravimetric (DTG), the first derivative of the TGA curve. In this study, the TGA/DTG curves, as a function of the temperature at a heating rate of 10 °C /min for a control sample and plastic films inoculated with strain NyZ600, were shown in Fig. 5. For the control sample, only single degradation stage occurred from 450 °C to 600 °C, where 72.2% of weight loss was observed in the form of volatile substances, and the maximum decomposition rate occurred at 510 °C. The weight loss occurred from 450 °C to 600 °C indicates the thermal decomposition of PC film. This result is consistent with the reported PC film weightlessness temperature, which occurs at the maximum weightlessness rate of thermal degradation (Delpech et al., 2002). In contrast, the strain NyZ600 inoculated plastic film showed two weight loss stages, stage 1 of 11.8% at 150–450 °C, stage 2 of 63.9% at 450–600 °C. The highest decomposition rates for these two

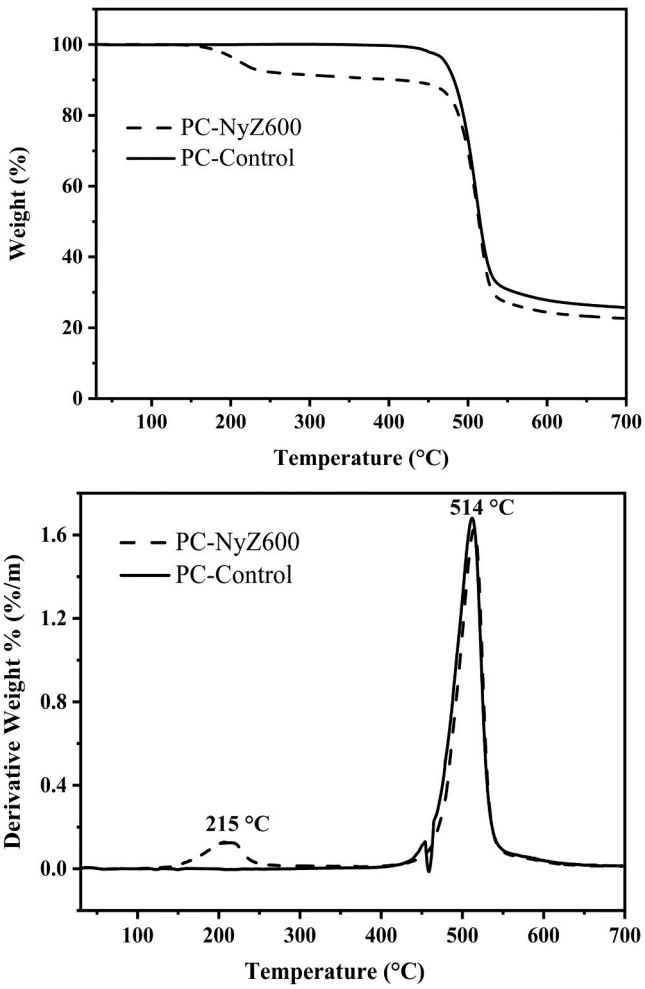

Fig. 5. Thermogravimetric analysis (TGA) (upper panel) and differential thermogravimetric (DTG) (low panel) curves of the control and the PC films treated by strain NyZ600 for 30 days.

weight loss stages occurred at 215 °C and 514 °C, respectively. The weight loss rate of strain NyZ600 inoculated plastic film was 75.7%, a slightly higher than that of the control group at 150–600 °C, suggesting poorer thermal stability of plastic film treated with bacteria. Decrease in thermal stability was thought to be the result of a decrease in the average molecular weight after degradation (Tsintzou et al., 2012). The aforementioned DSC curve between 150 and 300 °C exhibited distinct exothermic/endothermic areas from strain NyZ600 treated PC films, with temperature being 218.3 °C for the maximal endotherm, which is in consistent with the weight loss of 8.2% at 150–300 °C from the TGA curve. These results all suggest that the surface of the PC film after strain NyZ600 treatment was corroded and likely formed into lower molecular weight oligomers.

3.5. Decrease of molecular weights of PC polymer

The oligomers and its residual polymers are often analyzed by applying GPC to measure possible changes in molecular weight distribution (MWD) (Eubeler et al., 2009). Compared to the control sample, MWD shifted to lower molecular weights after treatment by strain NyZ600 (Fig. 6b). The Mw, Mn and Mz values for PC film inoculated with strain NyZ600 after 30 days were significantly lower than those for control samples. Mw value decreased from 45.67 ± 1.39 kDa to 31.97 ± 0.43 kDa; Mn value decreased from 23.55 ± 0.618 kDa to 16.75 ± 0.142 kDa. Mz value decreased from 73.44 ± 2.762 kDa to 51.40 ± 0.668 kDa (Fig. 6). Compared with the control samples, the

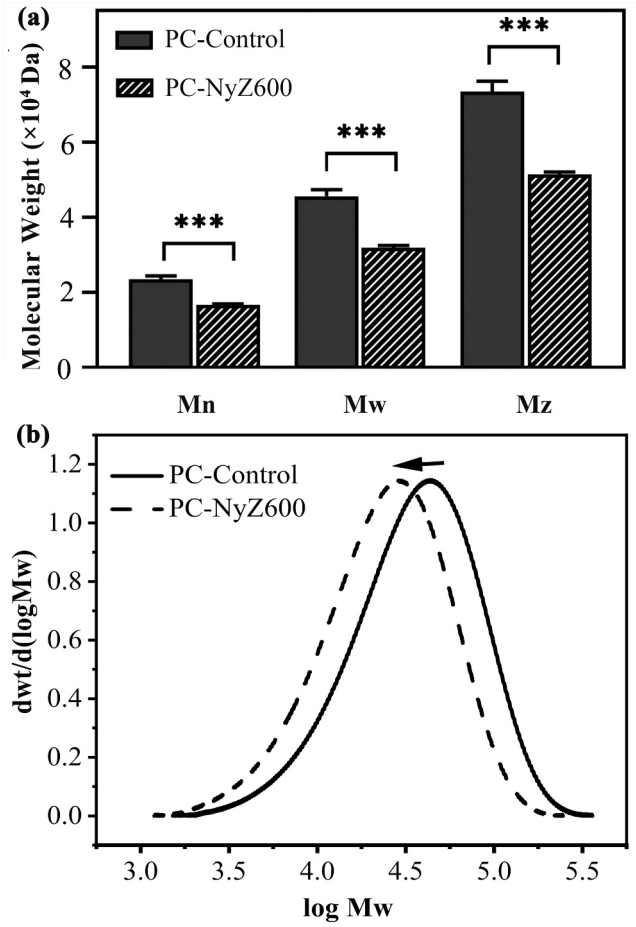

Fig. 6. Characterization of PC film degradation by gel permeation chromatography (GPC) (a). Bar diagrams show molecular weight changes in Mn, Mw and Mz. (Mean ± Standard Deviation, n = 3 for controls and samples treated by strain NyZ600, Significance (Student's *t*-tests) $p < 0.05$ indicated by *, $p < 0.01$ indicated by **, $p < 0.001$ indicated by ***). (b). Molecular weight distribution (MWD) of PC film of the control sample and the sample treated by strain NyZ600 sample. This curve shows the shift of PC plastic film treated with bacteria to the one with a lower molecular weight.

reduction rates for Mw, Mn and Mz were 30 ± 0.9%, 28.9 ± 0.6% and 30 ± 0.9% for the treated samples, respectively, showing a significant decreases in molecular weights after biotreatment. The GPC data indicated that the strain NyZ600 performed broad depolymerization of PC, that is, it decreases in three indicative parameters of Mn, Mw and Mz. The broad depolymerization of plastics was also described in recent research papers (Peng et al., 2020; Yang et al., 2021). Nevertheless, in contrast to a degradation study of UV pretreated PC film by using a commercial *Engyodontium album* MTP091 (Mn value decreased by 40% from 37.087 kDa to 22.24 kDa) (Artham and Doble, 2010), the Mn reduction rate in our study was somewhat lower (28.9%). This comparison may suggest that our PC degradation would have be enhanced if UV-pretreatment had been applied.

3.6. FTIR analysis and metabolite identification

At the end of the 30 days incubation period of PC film with the bacterial cells of strain NyZ600, evidences of PC depolymerization was obtained through ATR-FTIR analyses, as shown in Fig. 7. Previous FTIR analysis of PC degradation by esterases derived from eukaryotes showed that the carbonate bonds of polymers were reduced and hydroxyl groups were produced (Artham et al., 2011; Sivalingam and Madras, 2004b). Here, a relative decrease in the intensity of the carbonate carbonyl peak

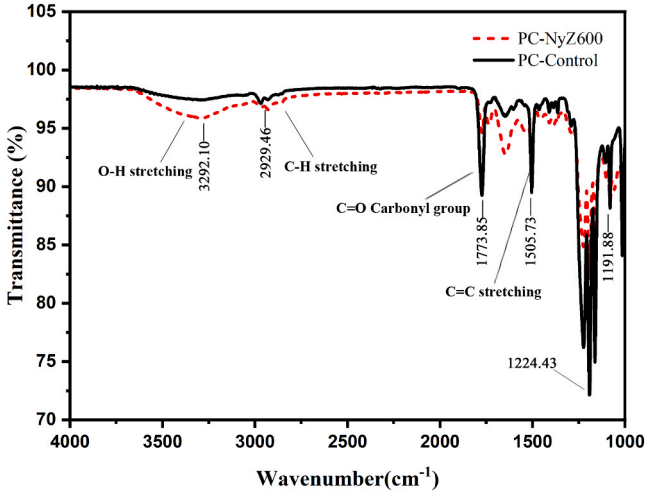

Fig. 7. Fourier transform infrared (FTIR) spectra of control sample (solid line) and PC plastic film treated with strain NyZ600 after 30 days (dashed line).

(at 1773 cm^{-1}) was observed and a characteristic peak of hydroxyl group near 3292 cm^{-1} was also emerged, which was also echoed by the observation of an increase in intensity of hydroxyl groups and a decrease in intensity of carbonate carbonyl groups during the XPS analysis. These indicated that PC film with bacterial treatment underwent the breakage of carbonate bonds and further formed phenyl hydroxyl compounds. According to the calculation of the bond dissociation energies, the weakest bond energy in the model compound of PC was the single bond between C-CH_3 (E_{B1}= 299.9 kJ/mol), and the dissociation energies of the second weakest bonds were the single key between the O-C=O (E_{B2}= 330.3 kJ/mol, E_{B3}= 337.8 kJ/mol) (Fig. 8a) (Huang et al., 2018). In addition, BPA and 4-cumylphenol (both are the monomers of PC) were detected and their identity confirmed by GC-MS, in comparison with the authentic compounds (Fig. 8, Figs. S4–5). It can be speculated that a specific and functional hydrolase secreted from strain NyZ600 broke the carbonate bonds of PC. In the process of PC synthesis, the chain extension of PC was terminated with 4-cumylphenol as capping agent (Okamoto, 2001). During the PC degradation, 4-cumylphenol may be initially produced by chain-end scission, and then the carbonate bond was broken to release BPA. Recently, it was thought that a breakdown of the polymer chain of plastics can be divided into two types: an exo-type and an endo-type degradation mechanism (Wei et al., 2019). The formation of 4-cumylphenol here suggested that an exo-type degradation mechanism was adopted in the PC film degradation by strain NyZ600. Among the previous studies of PC plastic films biodegradation, lipases derived from eukaryotes also acted, in an organic solvent, on the breaking of carbonate bonds to produce both 4-cumylphenol and BPA (Artham et al., 2011). However, no BPA was detected in reported fungal degradation of UV-pretreated PC films (Artham and Doble, 2010). In general, different degradation pathways were discovered in PC degradation by bacteria and fungi. The produced monomeric BPA from PC degradation can be re-used as a high-value-added chemical to reduce our dependence on fossil fuels.

Recent studies on bisphenol A have shown that bisphenol A can destroy the activity of bacterial cell membranes and hinder lipid synthesis by intercalating into bacterial cell membranes (Zaborowska et al., 2020). Therefore, the reason for the limited mass reduction of PC observed in our studies might be due to the certain toxic effect of bisphenol A on the inoculated strain NyZ600 in the process of the depolymerization. The removal of BPA in the culture may increase the weight loss rate of PC. However, this bacterium can not grow with PC as its sole carbon source, probably due to its incomplete catabolic pathway for PC degradation. On the other hand, a number of BPA utilizers have been isolated (Im and Löffler, 2016; Sasaki et al., 2005; Zhou et al.,

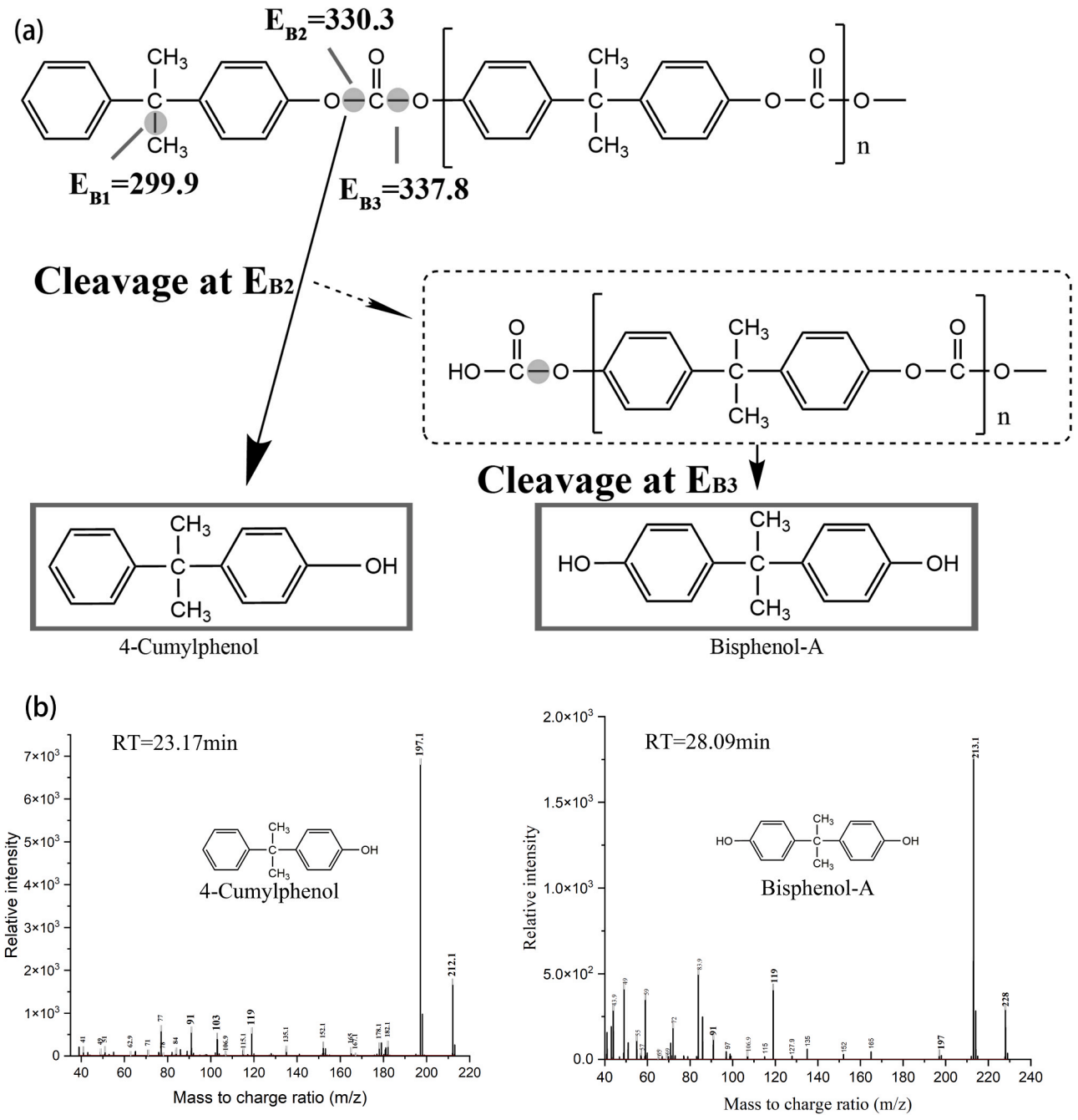

Fig. 8. Characterization of catabolites by gas chromatography-mass spectrometry (GC–MS) (a). Proposed catabolic reaction of PC plastic film degradation treated with strain NyZ600. The boxes with solid line mark the catabolites detected and the box with dotted line marks the proposed intermediate during the degradation. (b) Mass spectroscopy analysis of the catabolites from PC film degradation by strain NyZ600. A mass spectra from peaks with a retention time of 28.09 min and 23.17 min from GC-MS analysis (Fig. S4) in the total ion flow were identified as bisphenol A and 4-Cumylphenol respectively.

2015). This would make it possible to enhance PC remove by developing a complete catabolic pathway for PC degradation through the co-culture of strain NyZ600 and a BPA utilizer.

4. Conclusions and outlook

A bacterial strain, designated *Pseudoxanthomonas* sp. strain NyZ600, capable of degrading PC, was successfully isolated from activated sludge, by using a novel method in rapid screening for PC degrading bacteria from environmental samples. The treated PC films with strain NyZ600 were formed some "corrosion pits" on the PC film surface by observation of AFM and SEM. WAC results indicated that the inoculation of strain NyZ600 decreased the surface hydrophobicity and increased water-surface interaction of the PC film. The thermodynamic study of the PC film treated with strain NyZ600 showed that its thermal stability was poor. GPC observed that strain NyZ600 performed broad depolymerization of PC indicated by the reduction of Mn from 23.55 to 16.75 kDa and Mw from 45.67 to 31.97 kDa. FTIR and XPS characterization of the treatment PC film suggested that hydroxyl groups increased and carbonate carbonyl groups decreased. Two degradation products bisphenol A and 4-cumylphenol were also detected by GC-MS. All aforementioned evidences have reasonably established that strain NyZ600 was able to degrade the PC. This study has not only enriched the resource library for microbial degradation of PC but also comfortably

extended PC degraders from eukaryotic domain to prokaryotic domain, by using all analytic methods available.

Further research will focus on pre-treatment of PC plastics with aging technology and then degradation through strain NyZ600 may improve the efficiency of PC plastics removal. In addition, isolation and characterization of the specific depolymerase breaking the carbonate bond in PC will also be carried out, including cloning of its encoding gene. Ultimately, the depolymerase gene will be introduced into a BPA utilizer to create a recombinant strain capable of growing on PC films. This will provide a potential to enhance the biodegradation of PC films and microplastics in the future.

CRediT authorship contribution statement

Wenlong Yue: Writing-Original Draft, Conceptualization, Visualization, Investigation. **Chao-Fan Yin**: Conceptualization, Investigation. **Li min Sun**: Visualization, Writing- Reviewing & Editing. **Jie Zhang**: Formal analysis, Methodology. **Ying Xu**: Investigation, Visualization. **Ning-Yi Zhou**: Funding acquisition, Project administration, Supervision, Conceptualization, Writing - Original Draft , Reviewing & editing.

Declaration of Competing Interest

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

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2021.125775.

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