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Degradation of polyvinyl chloride by a bacterial consortium enriched from the gut of *Tenebrio molitor* larvae

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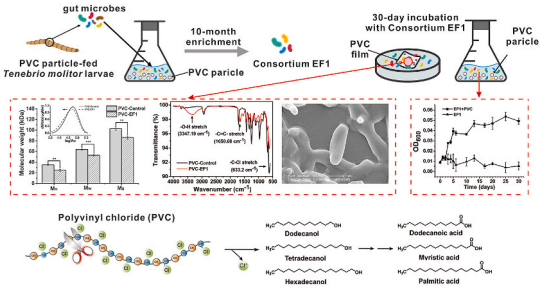
HIGHLIGHTS

- A bacterial consortium was enriched from gut microbiota of *Tenebrio molitor* larvae.
- The consortium utilized additive-free PVC as a sole carbon resource for its growth.
- Various analyses showed an efficient biodegradation of PVC by the consortium.
- Six intermediates of PVC biodegradation were identified by GC-MS.

GRAPHICAL ABSTRACT



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ABSTRACT

Polyvinyl chloride (PVC), a carbon backbone synthetic plastic containing chlorine element, is one of six widely used plastics accounting for 10% global plastics production. PVC wastes are recalcitrant to be broken down in the environment but release harmful chlorinated compounds, causing damage to the ecosystem. Although biodegradation represents a sustainable approach for PVC reduction, virtually no efficient bacterial degraders for additive-free PVC have been reported. In addition, PVC depolymerization by *Tenebrio molitor* larvae was suggested to be gut microbe-dependent, but to date no additive-free PVC degraders have been isolated from insect guts. In this study, a bacterial consortium designated EF1 was newly enriched from the gut of *Tenebrio molitor* larvae, which was capable of utilizing additive-free PVC for its growth with the PVC-mass reduction and dechlorination of PVC. PVC films inoculated with consortium EF1 for 30 d were analyzed by diverse polymer characterization methods including atomic force microscopy, scanning electron microscope, water contact angle, time-of-flight secondary ion mass spectrometry, Fourier transform infrared spectroscopy, differential scanning calorimetry, thermogravimetric analysis technique, and ion chromatography. It was found that bio-treated PVC films were covered with tight biofilms with increased –OH and –C═C- groups and decreased chlorine contents, and erosions and cracks were present on their surfaces. Meanwhile, the hydrophilicity of bio-treated films

Abbreviations: AFM, Atomic force microscopy; ATR-FTIR, Attenuated total reflection Fourier transform infrared spectroscopy; CFBAM, Carbon free basal agar medium; DSC, Differential scanning calorimetry; GC-MS, Gas chromatography-mass spectrometer; GPC, Gel permeation chromatography; LB, lysogeny broth; LCFBM, liquid carbon free basal medium; MM, mineral salts medium; PE, polyethylene; PP, polypropylene; PS, polystyrene; PVC, Polyvinyl chloride; SDS, Sodium dodecyl sulfate; SEM, Scanning electron microscopy; TGA, Thermogravimetric analysis; ToF-SIMS, Time-of-flight secondary ion mass spectrometry; WCA, Water contact angle.

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increased, but their thermal stability declined. Furthermore, M_w, M_n and M_z values were reduced by 17.0%, 28.5% and 16.1% using gel permeation chromatography, respectively. In addition, three medium-chain aliphatic primary alcohols and their corresponding fatty acids were identified as PVC degradation intermediates by gas chromatography-mass spectrometry. Combing all above results, it is clear that consortium EF1 is capable of efficiently degrading PVC polymer, providing a unique example for PVC degradation by gut microbiota of insects and a feasibility for the removal of PVC wastes.

1. Introduction

In the last century, synthetic plastics have been widely used in chemical industry, agriculture and daily life. The annual global plastics production totaled 367 million tons in 2020 (PlasticsEurope, 2021). Such high production and demand has resulted in an accumulation of huge amounts of plastic wastes in the environment, and this "white pollution" has become a serious environmental concern. Particularly, polyvinyl chloride (PVC), one of six widely used plastics, accounted for 10% global plastics production (PlasticsEurope, 2021). Additive-free PVC contains 56.8% (w/w) chlorine element with the chemical formula $[C_2H_3Cl]_n$. Due to its excellent mechanical and thermal properties, PVC with added plasticizer (10–70%) is widely used in various fields including construction engineering, packaging, medical treatment, electronics products, automotive and so on (PlasticsEurope, 2021). As a "C–C" inert structural backbone plastic, PVC wastes are recalcitrant to be broken down in the environment due to the nature of high molecular weight, highly stable covalent bond and highly hydrophobic, similar to polyethylene (PE) (Peixoto et al., 2017; Wei and Zimmermann, 2017; Yin et al., 2020). On the other hand, the traditional landfill and incineration approaches resulted in the release of harmful chlorinated compounds including hydrogen chloride and chlorinated dioxins (Glas et al., 2014; Pospisil et al., 1999; Reddy et al., 2010; Veronelli et al., 1999). Facing this increasingly serious "white pollution", developments of highly efficient and environmentally friendly plastic degradation methods are urgently required. Considering microbial versatile activities for catabolization and mineralization of xenobiotics (Alexander, 1981; Liu et al., 2021), degradation and recycling of plastics by microbes were considered feasible (Raddadi and Fava, 2019).

So far, several studies on the degradation of PVC by environmental fungi or bacteria have been reported. Seven white rot fungi (Kirbas et al., 1999) and *Cochliobolus* sp. from soil (Sumathi et al., 2016) were found to contain weak abilities to degrade the low molecular weight additive-free PVC. This degradation by white rot fungi was thought to be dependent on a nonspecific lignin degrading system (Kirbas et al., 1999), and the laccase in *Cochliobolus* sp. was verified to nonspecifically catalyze PVC oxidation (Sumathi et al., 2016). Four fungi isolated from the surface of thin additive-free PVC films buried in soil were all able to degrade PVC polymer with slight molecular weight reduction and surface topography changes (Ali et al., 2014). Furthermore, 20 fungal isolates from the surface of plasticized PVC exposed to the atmosphere all were able to grow with the plasticized PVC film, causing slight dry weight loss (max of 6.8%) (Webb et al., 2000). As to bacteria, two strains isolated from crude petroleum-oil contaminated soil were found to have the ability to degrade PVC containing epoxidized vegetable oil (75%, w/w) with tensile strength decreases and surface topography changes after an incubation period of 180 d (Das et al., 2012). Similarly, both *Pseudomonas citronellolis* DSM 50332 and *Bacillus flexus* DSM 1320 were able to degrade PVC films containing additives (35%, w/w) and thin packing bags with no more than 10% reduction of their weight-average molecular weight (M_w) after an incubation period of 90 d (Giacomucci et al., 2019). *Klebsiella* sp. EMBL-1 was also reported to be able to depolymerize and utilize additive PVC films (Zhang et al., 2022). In addition to these single strains, an anaerobic marine consortium was observed to form dense biofilms on the surface of additives-containing PVC films, resulting in weight loss (11.7 ± 0.6%), thermal stability decline and average molecular weight decrease of these films (Giacomucci et al., 2020). Although there are reported cases of microbes capable of degrading PVC listed above, the PVC plastics used in their degradation are largely plasticizers-containing ones, or the low degradation ability were found among the cases using additive-free PVC. It was considered that some strains mainly acted on PVC additives and only showed the low biodegradation rate of PVC polymer itself (Giacomucci et al., 2019). Furthermore, PVC mass reduction of compost-pretreated films treated by isolated bacterial strains was mainly due to the removal of additives (Novotny et al., 2022). Therefore, studies using additive-free PVC can provide solid evidences of PVC biodegradability.

In the past decade, *Tenebrio molitor* larvae (Coleoptera: Tenebrionidae) has been reported to effectively chew, ingest and degrade PE (Brandon et al., 2018; Wu et al., 2019; Yang et al., 2021), polystyrene (PS) (Wu et al., 2019; Yang et al., 2018) and PVC (Peng et al., 2020; Wu et al., 2019). Although PVC, PS and low density polyethylene (LDPE) plastic particles were all found to be degradable to some extent after they were feeding to *Tenebrio molitor* larvae for 30 d, PVC was found more difficult to be degraded than PE or PS (Wu et al., 2019). In another report when *Tenebrio molitor* larvae was fed with PVC plastic for 16 d, the values of M_w and number-average molecular weight (M_n) both decreased by at least 30% (Peng et al., 2020). Although plastics depolymerization by *Tenebrio molitor* larvae was suggested to be gut microbe-dependent (Peng et al., 2020; Yang et al., 2015a, 2020), only a limited bacterial strains from the gut of insects have been reported as possible PE degraders (Yang et al., 2014; Yin et al., 2020) and PS degraders (Wang et al., 2020; Yang et al., 2015a, 2015b), and no bacterial strains or consortia capable of degrading additive-free PVC have so far been isolated from insect guts. The depolymerization and degradation patterns of PVC by microbes still also remains unillustrated.

Here, we report a bacterial consortium capable of growing with additive-free PVC from the gut of *T. molitor* larvae. The changes of chemical and physical properties as well as the surface of the bio-treated PVC films were characterized using various approaches. The PVC degradation intermediates were also identified. This will enrich our understanding of the PVC degradation by cultivable bacteria from insect gut, and expand the resource library of microbial degradation of "C–C" inert structural backbone plastics. Meanwhile, this will also provide the evidences for revealing the possible microbial degradation pathway of PVC.

2. Materials and methods

2.1. Materials

Additive-free PVC powders [weight-average molecular weight (M_w): 62 kDa; number-average molecular weight (M_n): 35 kDa] (CAS No. 9002-86-2) and derivatizing reagent BSTFA-TMCS (99:1) were both purchased from Sigma-Aldrich Co. (St. Louis, MO). PVC powders (3 g) were dissolved in 100 ml of cyclohexanone (HPLC grade), and the solution (20 ml) was dropped on glass petri dishes (Φ 90 mm) before being placed in a fume hood at room temperature for 10 d. The PVC films were formed after the solvent being thoroughly evaporated. The PVC films were washed twice with 2% (w/v) SDS, 75% ethanol (v/v) and sterile water in turn, dried in the sterilized clean bench, and then cut into sheets of approximately 10 mm × 10 mm squares. The films were exposed to ultraviolet rays for 20 min to be further sterilized before being used as both test samples and negative controls. Additive-free PVC powders

were also processed as above to be sterilized before use.

2.2. Enrichment of PVC-degrading consortium

Tenebrio molitor larvae (sourced from Shandong Sishui-Limin Insect Breeding Plant, China) were fed with additive-free PVC powders by spraying water from time to time to maintain the humidity for two weeks. The gut suspensions of *Tenebrio molitor* larvae was collected as described previously (Yin et al., 2020). Then the obtained gut suspensions were moved into 100 ml liquid carbon free basal medium (LCFBM) (International, 1996) with sterilized additive-free PVC powders (1 g) on a rotary shaker (180 rpm) at 30 °C for the enrichment. After an incubation period of 30 d, the cultures (1%, v/v) were inoculated into 100 ml LCFBM with sterilized additive-free PVC powders (1 g) for another incubation of 30 d for a further enrichment. Since PVC was the sole carbon source during the enrichment, this process would theoretically lead to a sufficient population of possible PVC-degrading strains. After the enrichment cultivation repeating 10 times, a cultivable PVC-degrading consortium was obtained and designated EF1.

2.3. Measurement of the growth of consortium EF1 on PVC powders

Consortium EF1 was incubated in liquid lysogeny broth (LB) medium (Bertani, 1951, 2004) at 30 °C overnight to an optical density of 600 nm (OD_{600}) of approximately 0.6, and the cells were washed twice with LCFBM and resuspended in an equal volume of LCFBM. The cells solution (1%, v/v) was inoculated in 100 ml LCFBM with sterilized additive-free PVC powders (1 g) on a rotary shaker (180 rpm) at 30 °C. Above cells solution inoculated into the LCFBM without PVC powders were used as the negative control. The growth of consortium EF1 was monitored with the values of OD_{600} by a spectrometer (Lambda 25 UV/VIS, PerkinElmer). All experiments in this study were conducted in triplicate.

2.4. Detection of chloride ion during PVC biodegradation

LB-grown consortium EF1 were washed twice with liquid chlorine free mineral salts medium (chlorine free MM: $Na_2HPO_4{\cdot}12H_2O$ at 25.5646 g and KH_2PO_4 at 4.2694 g, along with the addition of 0.792 g $(NH_4)_2SO_4$, 0.28 mg $MnSO_4{\cdot}H_2O$, 0.3 mg $FeSO_4{\cdot}7H_2O$, 0.06 mg $MgSO_4{\cdot}7H_2O$, 1.23 mg $CaSO_4$, 0.05 mg $CuSO_4$, 0.05 mg $ZnSO_4$, 0.05 mg H_3BO_3, and finally, ultrapure water to a total volume of 1 L) and resuspended in an equal volume of chlorine free MM. The cells solution (1%, v/v) was inoculated in 10 ml chlorine free MM with sterilized additive-free PVC powders (1 g) on a rotary shaker (180 rpm) at 30 °C for an incubation period of 30 d. Sterilized additive-free PVC powders (1 g) immersed into chlorine free MM without inoculation served as the negative control. The culture suspension of the starting point and 30th d incubation (both 1 ml) were individually taken out for centrifugation at 8000×*g* for 20 min at room temperature. The supernatant was filtered through a 0.22-μm-pore membrane filter for determination of the chloride ion concentration by ion chromatography (IC) (ICS-5000+/900, Thermo Fisher Scientific, U.S.A.).

2.5. Characterization of PVC-mass reduction and molecular weight distribution of PVC films

Cells of consortium EF1 were prepared as the aforementioned method and spread across carbon free basal agar medium (CFBAM: LCFBM added 5% (w/v) agar) (Yin et al., 2020) plates, and then covered with pieces of sterilized PVC film. Sterilized PVC films covering on CFBAM plates without inoculation served as the negative control. After an incubation period of 30 d at 30 °C, all the residual PVC films treated by consortium EF1 and the negative control were collected, washed twice with 2% (w/v) SDS, 75% ethanol (v/v) and sterile water in turn for thoroughly removing extracellular secretions or biofilms on the film surfaces.

Sterilized PVC films (10 mm × 10 mm) were measured for their initial dry weights before being covered on the plates. The dry weights of residual PVC films were measured after films being washed and dried thoroughly at room temperature. The PVC-degrading ability according to PVC-mass reduction (w/w) was expressed as [(initial PVC dry weight - residual PVC dry weight)/initial PVC dry weight × 100%].

For gel permeation chromatography (GPC) analysis, the aforementioned thoroughly washed residual PVC films (10 mg) were dissolved in tetrahydrofuran (10 ml). The solution was filtered through a 0.22-μm-pore membrane filter for use. Sterilized PVC film without inoculation served as the negative control. The molecular weights of samples and the negative control were analyzed using GPC (HLC-8320GPC, Tosoh Corp, Japan) as described previously (Yue et al., 2021). Polydispersity index (PDI), a measure of the distribution of molecular mass in polymer diversified indication, was calculated as PDI = M_w/M_n as described previously (Peng et al., 2020).

2.6. Topographical, physical and chemical characterizations of PVC films surface

PVC films (10 mm × 10 mm) treated by consortium EF1 were prepared as the aforementioned method, and sterilized PVC films (10 mm × 10 mm) without inoculation served as the negative control.

PVC films were peeled off carefully from CFBAM plates. Some films were air-dried adequately and coated with Au for the observation of biofilms formed by consortium EF1, and the deterioration of PVC films surface using scanning electron microscopy (SEM) (FESEM, Sirion 200, FEI, U.S.A.) as previously described (Yin et al., 2020) with modifications. The other ones were thoroughly washed twice to remove extracellular secretions or biofilms on the film surfaces and dried as the aforementioned method, then were used for the detection of the deterioration and the water contact angles of PVC films surface by atomic force microscopy (AFM) (Multimode NanoscopeIIIa, Bruker, Germany) and a video-based optical contact angle instrument (DSA100, Kruss, Germany), respectively, as described previously (Yue et al., 2021) with modifications.

The formation of new functional groups of PVC films (being thoroughly washed and dried without attachments and H_2O) were characterized by attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR) (Nicolet 6700, Thermo Fisher Scientific, U.S.A.) in the frequency range of 4000–500 cm^{-1} as previously described (Peng et al., 2020; Yin et al., 2020; Yue et al., 2021). The surface chemical compositions of the PVC films (being thoroughly washed and dried without attachments and H_2O) were analyzed by time-of-flight secondary ion mass spectrometry (ToF-SIMS) (TOFSIMS.5–100, ION-TOF GmbH, Germany). Bi_3^+ 30 keV ions and GCIB were used as spectral analysis and sputter beam, respectively. The Bi_3^+ current was 1.142 pA. Image of a single film sample was acquired using Bi_3^+ 30 keV ions rastering over a 56 × 34 μm^2 area with 128 × 128 pixels in normal 2D image mode. Charge compensation was achieved with the pulsed low energy electron flood gun. The presented spectra were normalized to total ions.

2.7. Thermal characterizations of PVC films

PVC films peeled off from CFBAM plates after an inoculation 30 d were thoroughly washed twice to remove extracellular secretions or biofilms on the film surfaces and dried as the aforementioned method for the thermal investigations. Sterilized PVC films without inoculation served as the negative control.

Thoroughly washed PVC films (approximately 10 mg) were subjected to the thermogravimetric analysis (TGA) using a thermal analyzer (Pyris-1 TGA, PerkinElmer, U.S.A.) under a high-purity nitrogen atmosphere. The thermograms were recorded from 30 to 700 °C at a heating rate of 10 °C/min. Glass transition temperature (T_g) of PVC films

(approximately 5 mg) was detected using differential scanning calorimeter (DSC) (DSC 204 F1, NETZSCH, Germany) as described previously (Yue et al., 2021) with modifications. T_g values were taken from the second heating process (Yu et al., 2016; Zampino et al., 2021), and estimated by the mid-Cp interpolation method (Tsintzou et al., 2012).

2.8. Characterization of intermediates by gas chromatography-mass spectrometer (GC-MS)

PVC films (10 mm × 10 mm) covering on CFBAM plates with an inoculation of 30 d were peeled carefully from CFBAM. The plates and PVC films were washed twice with sterile water (5 ml) and the cleaning solution was collected. The possible metabolites from PVC film degradation were extracted twice from the cleaning solution with equal volume of dichloromethane. The resulting organic phases were dehydrated with anhydrous Na_2SO_4, and further concentrated by revolving evaporation. Next, the possible metabolites were dissolved with 1 ml pure dichloromethane and incubated with derivatizing reagent BSTFA-TMCS at 60 °C for 30 min. Then the solution filtered through a 0.22-μm-pore nylon membrane filter was used for PVC degradation intermetabolites detection by gas chromatography-mass spectrometer (GC-MS) system (Agilent, U.S.A.) consisting of a 7890B-GC with MMI inlet and 5977B-MS with a quadrupole mass spectrometer as described previously (Yue et al., 2021).

2.9. Microbial community analysis of consortium EF1

Cells of consortium EF1 were prepared as aforementioned. The microbial DNA was extracted using the E.Z.N.A.® soil DNA Kit (Omega Biotek, Norcross, GA, U.S.A.) The V3–V4 hypervariable region of the bacterial 16S rRNA gene was amplified with primers 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and 806R (5′- GGACTACHVGGGTWTCTAAT-3′).

Phasing amplicon sequencing was carried out to sequence the amplified V3–V4 region of 16S rRNA gene, and purified amplicons were pooled in equimolar and paired-end sequenced (2 × 300) on an Illumina MiSeq platform (Shanghai Personal Biotechnology Co., Ltd.). Sequencing data (SRA accession number SRR16679935) were demultiplexed, quality-filtered and then merged based on the criteria, and chimeric sequences were identified and removed applying DADA2. Operational Taxonomic Units (OTUs) were clustered with 97% similarity cutoff using software QIIME2 (2019.4). Finally, taxonomy of each 16S rRNA gene sequence was analyzed by RDP Classifier against the Silva (SSU123) 16S rRNA database using confidence threshold of 70%.

3. Results and discussion

3.1. Enrichment and diversity of PVC degrading bacterial consortium

After ten cycles of enrichment with additive-free PVC powders as the sole carbon source, a bacterial consortium designated EF1 was enriched. According to relative abundance analysis based on V3–V4 region of 16S rRNA gene amplicons, consortium EF1 largely consisted of strains belonging to ten genera (*Stenotrophomonas* (about 78%), *Enterococcus* (about 15%), *Acinetobacter* (about 2%), *Xylella*, *Pseudochrobactrum*, *Ochrobactrum*, *Lactobacillus*, *Streptococcus*, *Bacillus*, and others (about 4%)) in ten families (Xanthomonadaceae (about 80%), Enterococcaceae (about 18%), Moraxellaceae (about 2%), Brucellaceae, Aeromonadaceae, Streptococcaceae, Lactobacillaceae, Rhizobiaceae, Bacillaceae, and Yersiniaceae) of four phyla (Proteobacteria (about 82%), Firmicutes (about 18%), Actinobacteria, and Spirochaetes) (Fig. S1).

3.2. Growth on additive-free PVC and the dechlorination of PVC

To explore the possibility of growth for the enriched consortium with PVC, consortium EF1 was grown with additive-free PVC powders in LCFBM. The growth curves (Fig. 1a) indicated that consortium EF1 was able to grow on additive-free PVC powders in LCFBM, and the biomass increased to five times high comparing with the initial one after an incubation of 30 d. However, there was no growth when consortium EF1 was incubated without PVC. These results indicated that consortium EF1 was able to utilize additive-free PVC as the sole carbon and energy for its growth.

To detect the change of chloride concentration in the culture incubated with PVC, consortium EF1 was cultured with additive-free PVC powders in chlorine free MM. As shown in Fig. 1b, after an incubation period of 30 d, the chloride concentration in the cell supernatant (4.65 ± 0.82 mg/L) was approximately 2-fold to the initial one (2.43 ± 0.06 mg/L), while no change in chloride concentration was observed in the negative control without consortium EF1 inoculation. These results indicated that a dechlorination accompanied the utilization of additive-free PVC powders by consortium EF1.

3.3. PVC-mass reduction and decreases in molecular weight of PVC films treated by consortium EF1

To assess the PVC-mass reduction of films during the degradation by consortium EF1, the changes in dry weight of PVC films were measured after an inoculation of 30 d. As shown in Fig. 2a, 6.13% of PVC was removed, but the loss in PVC films without biotreatment was less than 1%. This PVC-mass reduction resulted from the biotreatment is consistent with the aforementioned growth of consortium EF1 with additive-free PVC.

Possible changes in molecular weight distribution of polymers are often measured by gel permeation chromatography (GPC), an essential tool to study their depolymerization or degradation (Eubeler et al., 2009). In general, M_n (number-average molecular weight) provides information about the lower molecular weight fraction, M_w (weight--average molecular weight) is the average closest to the center of the distribution curve, and size-average molecular weight (M_z) represents the higher molecular weight portion (Wu and Criddle, 2021). As shown in Fig. 2b, compared to the negative control, the molecular weight distribution of PVC films treated by consortium EF1 for 30 d shifted to lower molecular weights. Their M_w, M_n and M_z values were significantly lower than those untreated. M_w value decreased from 63.71 ± 1.03 kDa to 52.86 ± 1.40 kDa; M_n value decreased from 34.75 ± 0.82 kDa to 24.83 ± 1.98 kDa; M_z value decreased from 102.81 ± 3.41 kDa to 86.23 ± 2.61 kDa. The reduction values of M_w, M_n and M_z clearly indicated significant decreases in molecular weights after bio-treatment, which were considered to be the credible evidences of PVC degradation in insect gut (Peng et al., 2020; Wu and Criddle, 2021). Particularly, the reduction proportion of M_w (28.5%) was significantly higher than those of M_n and M_z (17.0% and 16.1% respectively). Meanwhile the PDI (=M_w/M_n) of the residual PVC (2.13) was also higher than that of PVC without inoculation (1.83). These results indicated a broad extent depolymerization of the polymer chains in the bio-treated PVC.

3.4. Changes in topographical and physical properties of bio-treated PVC films surfaces

Atomic force microscopy (AFM) observation was applied to detect surface topography and roughness of the PVC films with inoculation of consortium EF1 for 30 d. As shown in AFM images (Fig. 3), compared with the negative control without inoculation, there were obvious topographic changes in the bio-treated PVC films (being thoroughly washed without extracellular secretions or biofilms) with increased values of Ra (5.319 nm in bio-treated film and 2.069 nm in negative control), Rq (9.979 nm in bio-treated film and 2.593 nm in negative control), and Rz (131.4 nm in bio-treated film and 24.25 nm in negative control). The significant increases in all Ra, Rq, and Rz values indicated the evident changes in the topography and roughness of the surface of PVC films after biodegradation, and a similar phenomenon was also

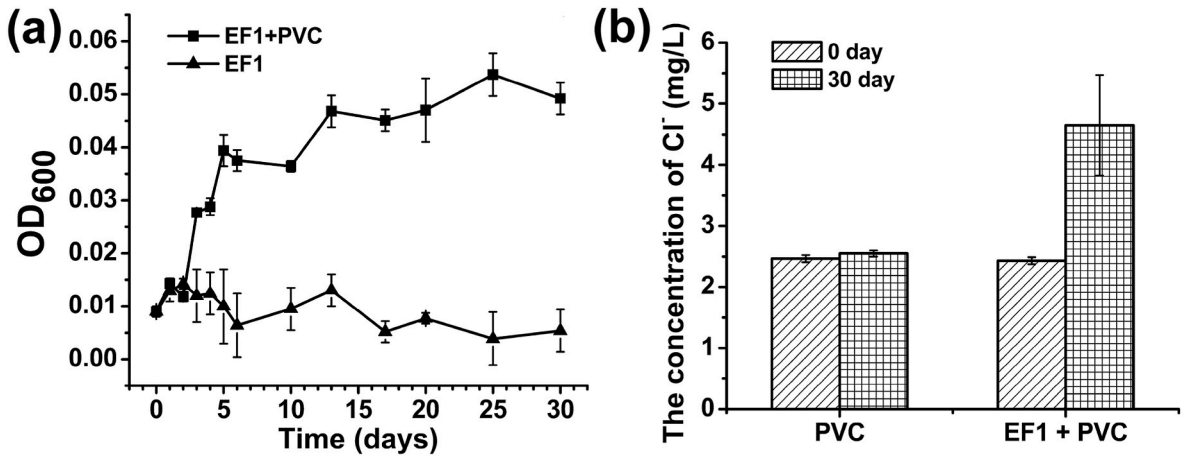

Fig. 1. Growth of consortium EF1 on additive-free PVC with dechlorination. (a) Growth curves of consortium EF1 on additive-free PVC particle (n = 3). EF1: consortium EF1 cultured without carbon source; EF1+PVC: consortium EF1 cultured with additive-free PVC powders. (b) Changes of chlorination concentration in the cell supernatant (n = 3). PVC: additive-free PVC powders in chlorine free MM without inoculation; EF1+PVC: consortium EF1 cultured with additive-free PVC powders in chlorine free MM.

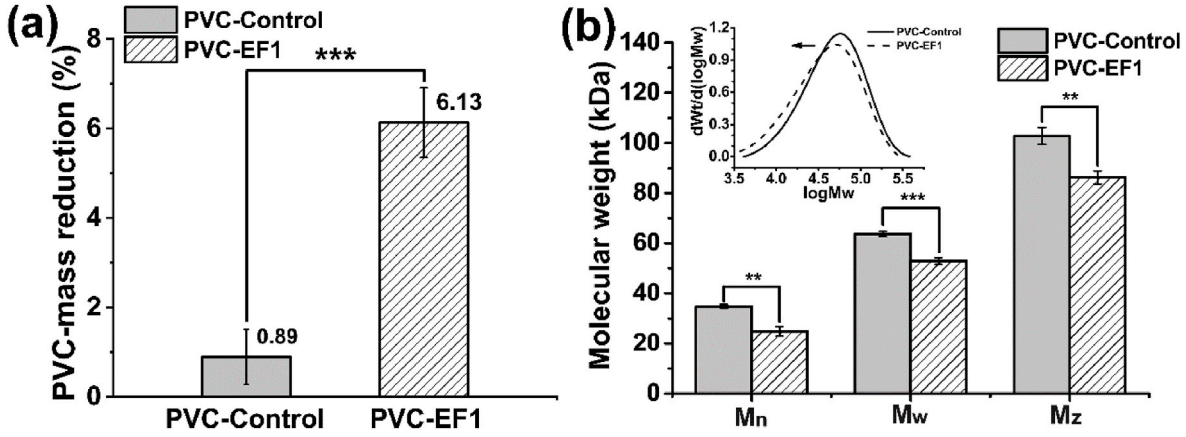

Fig. 2. PVC-mass reduction and molecular weight distribution decrease of bio-treated PVC films. (a) PVC-mass reduction of films treated by consortium EF1 (n = 3, Significance, $p < 0.001$ indicated by ***). (b) Characterization of PVC film degradation by GPC. Bar diagrams show molecular weight changes in values of M_n, M_w and M_z (n = 3; Significance, $p < 0.01$ indicated by **, $p < 0.001$ indicated by ***). The inserted line-point figure shows molecular weight distribution of PVC films. PVC-Control: PVC films without inoculation; PVC-EF1: PVC films treated by consortium EF1.

observed during PE (Peixoto et al., 2017) and PC (Yue et al., 2021) degradation by bacteria. Particularly, significant increase in the Rz values of bio-treated PVC films indicated the formation of wide and deep cavities as explained previously (Peixoto et al., 2017).

Meanwhile, the colonization of the consortium EF1 and cracks on the surface of PVC films were both observed using scanning electron microscope (SEM). It can be seen that consortium EF1 adhered on the PVC surface to form a tight biofilm, and the serious cracks on the PVC film surfaces occurred after being treated with the consortium (Fig. 4 c and d). As to the negative control without inoculation, no colonies were present on the film, and its surface was still smooth and the dense structure remained unchanged (Fig. 4 a and b). Commonly biofilm formation is a useful strategy for bacteria to colonize on the hydrophobic plastic films to be supportive for their growth (Yin et al., 2020). Tight biofilms were also observed during bacterial degradation of PE (Mohanrasu et al., 2018; Yin et al., 2020) and plasticized PVC (Das et al., 2012). And the secretion of bacterial extracellular enzymes were suggested to result in the cracks on the surface of plastics (Yoshida et al., 2016).

Water contact angle (WCA), one of the evaluation criteria for the surface properties of materials, is commonly measured to determine the changes in hydrophobicity on polymer surfaces (Wu and Criddle, 2021). The values of contact angle indicated the surface properties of plastic polymers being hydrophobic (contact angle >90°) or hydrophilic (contact angle <90°) (Peters, 2017). As shown in Fig. 5a, the contact angles of the PVC film (being thoroughly washed without extracellular secretions or biofilms) treated by consortium EF1 were measured to be 74.43 ± 1.48° (n = 3), which significantly decreased comparing with the negative control (90.92 ± 2.79°; n = 3). The significant decrease ($P < 0.05$) in contact angle values (about 16°) indicated that the hydrophilicity of bio-treated PVC increased significantly, apparently resulted from the aforementioned roughness and cracks occurred on the surfaces of the biotreated PVC films. A similar phenomenon was also observed during PS (Woo et al., 2020) or PC (Yue et al., 2021) degradation by bacteria.

Therefore, all the above results about changes in topographical and physical properties of the PVC films surface indicated that the degradation and depolymerization of PVC was indeed occurring with the treatment by consortium EF1.

3.5. Changes in chemical properties of biotreated PVC films surfaces

Attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR) was used to reveal the possible chemical modifications on

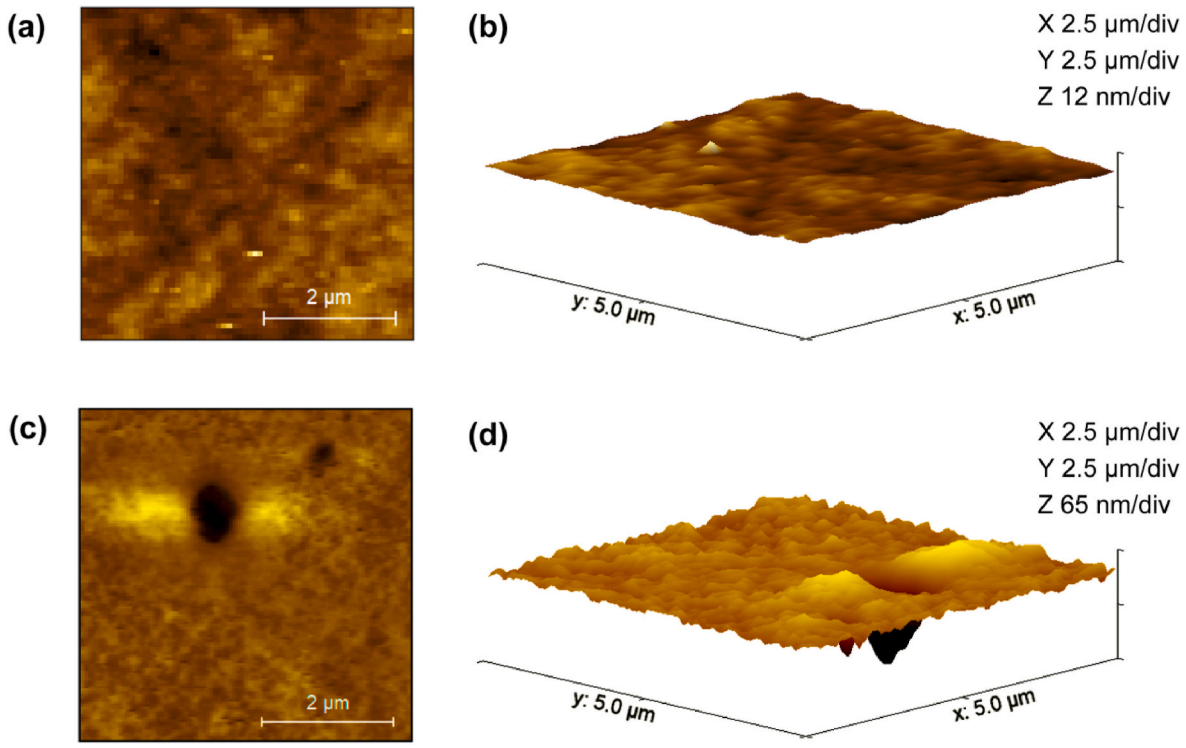

Fig. 3. Two dimensional (2D) and three dimensional (3D) AFM images of the physical surface topography of PVC films. (a) 2D and (b) 3D images of the negative control sample; (c) 2D and (d) 3D images of the PVC films treated by consortium EF1.

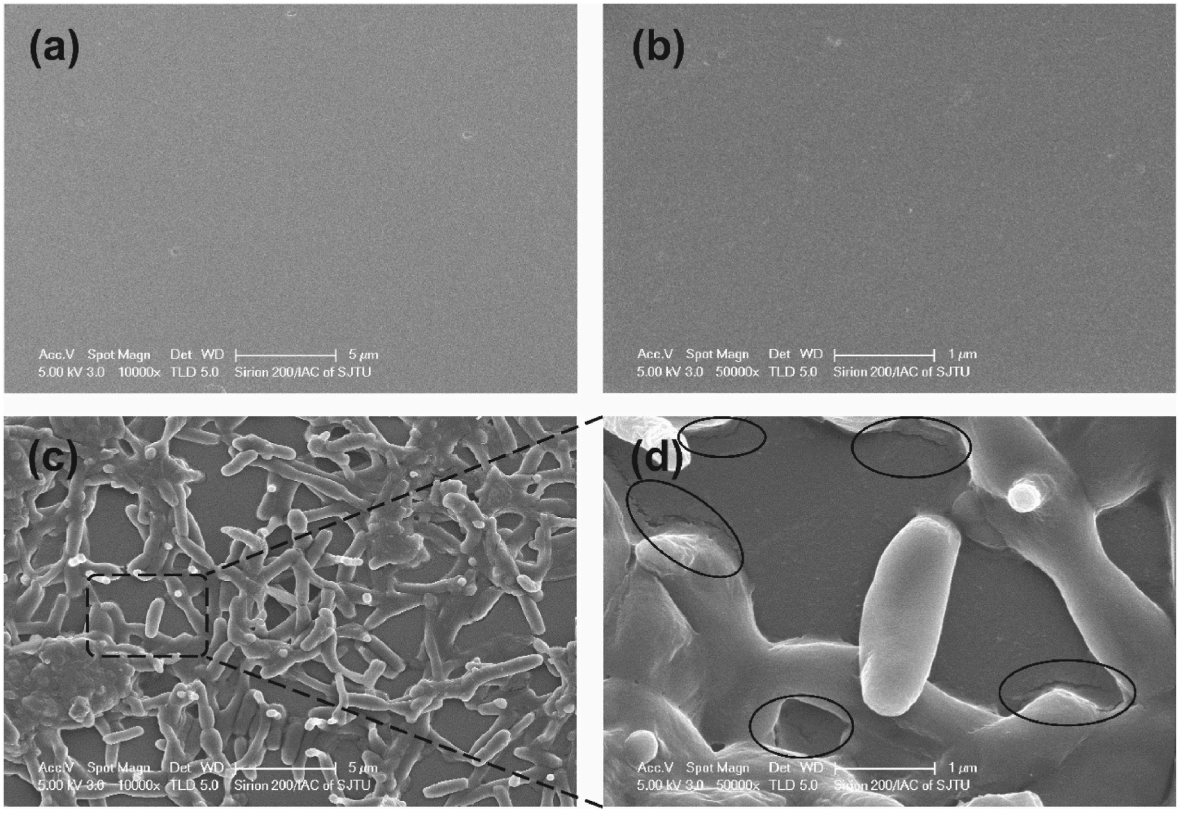

Fig. 4. Scanning electron microscopy (SEM) images of the negative control sample (a and b) and the bio-treated PVC film (c and d). A part in the dashed box at scale of 5 μm (c) represents the whole image at scale of 1 μm (d). The ellipses indicate the presence of cracks around bacterial cells on the film surface after the bio-treatment.

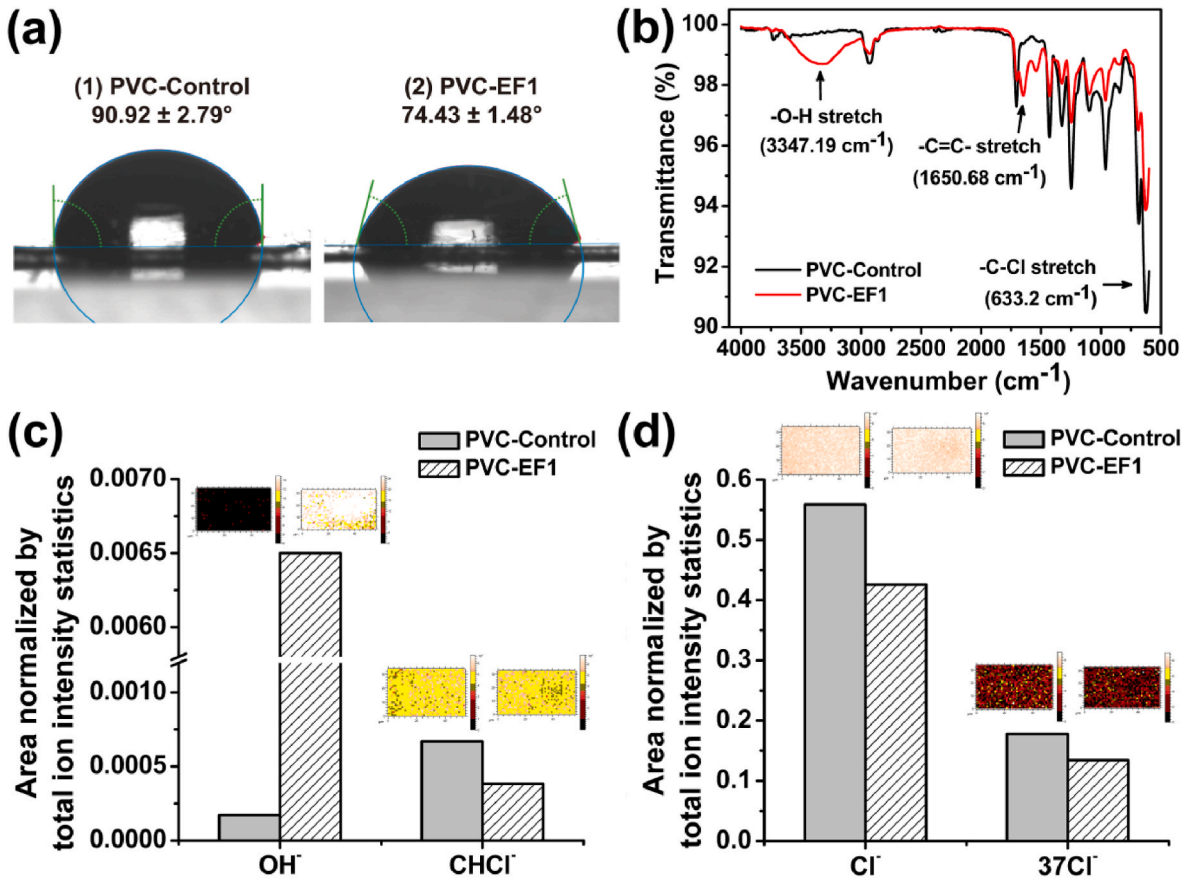

Fig. 5. Characterization of physical and chemical properties of the surface of PVC films. (a) Water contact angle (WCA) values of the bio-treated PVC film and the negative control sample (n = 3). (b) FTIR spectra of the bio-treated PVC film (red line) and the negative control sample (black line). (c) Areas normalized by total ion intensity statistics of OH^- and $CHCl^-$, illustrating the concentration changes of OH^- and $CHCl^-$ on the PVC films surfaces. (d) Areas normalized by total ion intensity statistics of Cl^- and $^{37}Cl^-$, illustrating the concentration changes of Cl^- and $^{37}Cl^-$ on the PVC films surfaces. The inserted color shading pictures by the ion imaging mass spectrometry indicated a change in the magnitude of the ion count values at different sites and was a reflection of the change in the concentration distribution of specific ion composition in the microzone. PVC-Control: PVC films without inoculation; PVC-EF1: PVC films treated by consortium EF1. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

the surface of PVC films treated by consortium EF1 for 30 d. Compared to the negative control without inoculation, the treated PVC film (being thoroughly washed without extracellular secretions or biofilms) was featured by two new peaks corresponding to those of the -O-H bond (3347.19 cm^{-1}) and the –C═C- bond (1650.68 cm^{-1}) (Fig. 5b), indicating the occurrence of oxidation on PVC surfaces. Meanwhile, the peak representing the -C-Cl bond (633.2 cm^{-1}) became significantly weaker than that in the negative control (Fig. 5b), which confirmed dechlorination accompanying the biodegradation. The similar phenomenon was also observed during PVC degradation by *T. molitor* larvae (Peng et al., 2020).

Time-of-flight secondary ion mass spectrometry (ToF-SIMS) was also employed to verify the changes in chemical composition of the PVC film surface treated by consortium EF1 for 30 d. The ToF-SIMS spectra of the area normalized by total ion intensity statistics of OH^- and $CHCl^-$, Cl^- and $^{37}Cl^-$ are shown in Fig. 5c and d, respectively. Compared to that of the negative control without inoculation, the normalized area of OH^- of the bio-treated sample (being thoroughly washed without extracellular secretions or biofilms) increased by 37-fold to 6.50×10^{-3} from 1.71×10^{-4} (Fig. 5c). Meanwhile the obvious change in color from dark to light also indicated that the content of OH^- increased significantly at different sites in the microzone of the treated sample surface as shown in Fig. 5c. These results were consistent with the aforementioned newly emerged peak of the -O-H bond on the surface of bio-treated PVC films by ATR-FTIR analysis (Fig. 5b), and also proved the occurrence of oxidation on surfaces of bio-treated PVC films. In contrast to the negative control, the chlorine content ($CHCl^-$, Cl^- and $^{37}Cl^-$) was also altered, with normalized areas of $CHCl^-$, Cl^- and $^{37}Cl^-$ decreasing from 6.70×10^{-4} to 3.83×10^{-4} (42.8% reduction) (Figs. 5c), 5.59×10^{-1} to 4.26×10^{-1} (23.8% reduction) (Figs. 5d) and 1.78×10^{-1} to 1.34×10^{-1} (24.7% reduction) (Fig. 5d), respectively. Particularly based on the changes of the normalized areas, the contents of Cl^- and $^{37}Cl^-$ declined with a similar proportion. These results were consistent with the aforementioned appearance of chloride ions in the supernatant (Fig. 1b) and the reduced peak of -C-Cl bond (633.2 cm^{-1}) on the surface of bio-treated PVC films (being thoroughly washed without extracellular secretions or biofilms) by ATR-FTIR analysis (Fig. 5b), indicating the occurrence of the dechlorination during the PVC degradation.

Therefore, all the above results about changes in chemical properties of the PVC films surface (being thoroughly washed without extracellular secretions or biofilms) indicated that the degradation and depolymerization of PVC was occurring with the treatment by consortium EF1.

3.6. *Thermal stability reduction of biotreated PVC*

The decline of thermal stability indicates the degradation of plastics (Giacomucci et al., 2019). Thus thermogravimetric analysis (TGA), a thermal analytic technique (Wu and Criddle, 2021), was employed for the thermal properties analysis of bio-treated PVC.

The thermal degradation behavior of the PVC films was investigated by TGA and differential thermogravimetric (DTG, the first derivative of the TGA curve). Bio-treated PVC films (being thoroughly washed

without extracellular secretions or biofilms) and the negative control with inoculation both showed three weight loss stages (Fig. 6a). The first stage was attributed to the decomposition of residual solvent cyclohexanone (stage 1), and the other two stages were associated with the release of hydrogen chloride (stage 2), and the pyrolysis of the C–C backbone chain (stage 3). Omitting stage 1, two maximum decomposition rates of bio-treated PVC polymer in stage 2 and stage 3 occurred at 283.13 °C and 482.4 °C, respectively, which decreased compared with those of the negative control (at 285.54 °C and 484.96 °C, respectively) (Fig. 6a). The similar phenomenon was also observed during PC degradation by bacteria (Yue et al., 2021) and PVC degradation by *Tenebrio molitor* larvae (Peng et al., 2020). Because the decrease of the thermal stability can be used as an indication for plastics biodegradation (Giacomucci et al., 2019), the above results suggested that bio-treated PVC films were degraded, likely forming lower molecular weight oligomers with different thermal properties.

3.7. Glass transition temperature (T_g) decrease of PVC films by differential scanning calorimetry analysis

To investigate the changes in thermal transition of PVC with or without treatment by consortium EF1, the glass transition temperature (T_g) of them was detected using differential scanning calorimetry (DSC). If T_g migrates to low temperature, it is usually related to the decline of plastic stability caused by degradation (Lucas et al., 2008). In this study, corresponding to the midpoint of the DSC curves measured from the extension of the pre- and post-transition baseline, T_g of the negative control and the EF1-treated PVC film (being thoroughly washed without extracellular secretions or biofilms) were 42.15 °C and 36.13 °C, respectively (Fig. 6b). A decrease of 6.02 °C in T_g value between the negative control and the bio-treated PVC film was observed, an indication of the decrease in the average molecular weight caused by depolymerization as explained previously (Tsintzou et al., 2012), resulting from PVC degradation by consortium EF1. A similar phenomenon was also observed during PC degradation by a bacterial strain (Yue et al., 2021). It is worth noting that the T_g values in this study were lower than the theoretical T_g value of additive-free PVC (Yu et al., 2016). This might be resulted from the residual solvent cyclohexanone which likely acted as a plasticizer for increasing the free volume and distance of PVC chains and then efficiently decreasing the T_g value, analogues to a report about T_g value reduction where propargyl ether cardanol was used an additive for PVC plasticization (Jia et al., 2017).

3.8. Intermediates identification of PVC degradation

Gas chromatography-mass spectrometry (GC-MS) was employed to identify intermediates of PVC degradation by consortium EF1. As shown in Fig. 7b and Fig. S2, six compounds with retention time of 17.572, 18.328, 19.571, 20.477, 21.614 and 22.440 min were identified as dodecanol (C12), dodecanic acid (C12), tetrodecanol (C14), myristic acid (C14), hexadecanol (C16) and palmitic acid (C16), respectively. Compared with the negative control, the amounts of dodecanol, dodecanic acid and tetrodecanol all increased significantly, and those of hexadecanol and palmitic acid both increased slightly, whereas myristic acid was a newly emerged compound (Fig. S2). The above three medium-chain aliphatic primary alcohols and their corresponding medium-chain fatty acids were considered as the possible intermediates of PVC degradation in this study (Fig. 7b). The presence of increased or newly emerged intermediates suggested the occurrence of depolymerization and degradation of PVC with the treatment by consortium EF1. However neither chlorinated alcohols nor chlorinated organic acids were detected by GC-MS in this study. To date, little is known about dechlorination of long-chain chlorinated organics under aerobic or anoxic environment. Aerobic dechlorination of short-chain chlorinated alcohols or acids could be conducted with bacterial dehydrohalogenases (Heeb et al., 2019). In addition, as much as 62.51% Cl element was able to be accumulated in unknown chlorinated organic matters in the frass of *T. molitor* larvae fed with PVC (Peng et al., 2020). It is likely that, in our case, the dechlorination occurred along with the formation of intermediates following the cleavage of PVC polymer chains.

In previous reports, PVC biodegradation was suggested to involve at least three reactions: (a) depolymerization or cleavage of polymer chains; (b) formation of oxidized intermediates along with Cl^- release, and (c) mineralization of intermediates to CO_2 and H_2O (Peng et al., 2020; Wu and Criddle, 2021). Based on the intermediates detected by GC-MS in this study and the previous reports, a degradation pathway of PVC by consortium EF1 was proposed as shown in Fig. 7a. Firstly PVC chain was oxidized and broken down to produce aliphatic primary alcohols (including dodecanol, tetrodecanol and hexadecanol) with dechlorination. This reaction probably was catalyzed by the nonspecific oxidases, such as laccase (Sumathi et al., 2016) or other nonspecific lignin degrading system (Kirbas et al., 1999). After PVC polymer chains being broken down, Cl^- release was catalyzed by dehydrohalogenases. Then formed primary alcohols were further oxidized to the corresponding aldehydes and fatty acids (including dodecanic acid, myristic acid and palmitic acid) by corresponding alcohol dehydrogenase and aldehyde dehydrogenase respectively. Finally the resulting acids were degraded via β-oxidation pathway. Petroleum-based plastics including PE, PS, PP and PVC share similar backbone structure with alkanes. It was suggested here that the medium-chain intermediates (such as dodecanol, tetrodecanol and hexadecanol) formed by the cleavage of these plastics may further entered a degradation pathway similar to that of alkane degradation (Rojo, 2009).

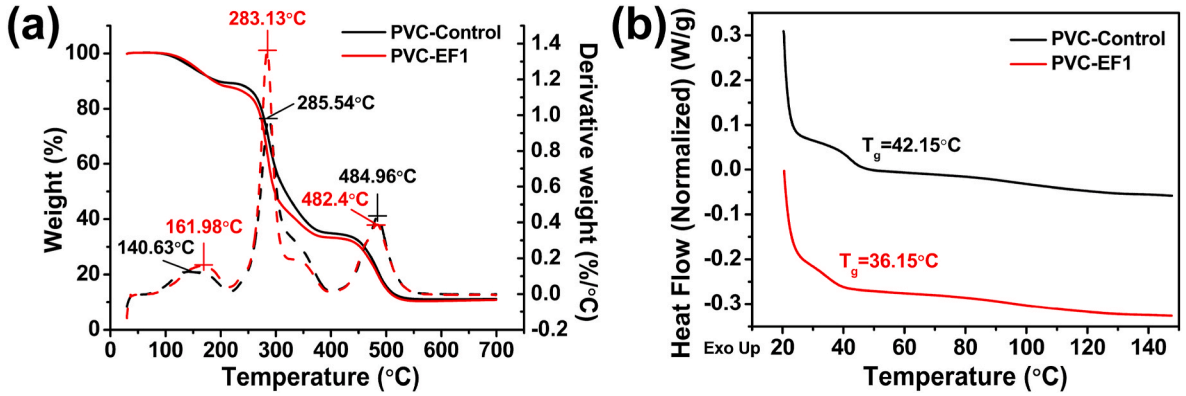

Fig. 6. Detection of thermodynamic properties of PVC films. (a) TGA spectra of the bio-treated PVC film and the negative control. The weight curve was drawn in solid lines (left axis) while the derivative weight curve was drawn in dash lines (right axis). (b) DSC curves from the second heating process of the bio-treated PVC film and the negative control. PVC-Control: PVC films without inoculation; PVC-EF1: PVC films treated by consortium EF1.

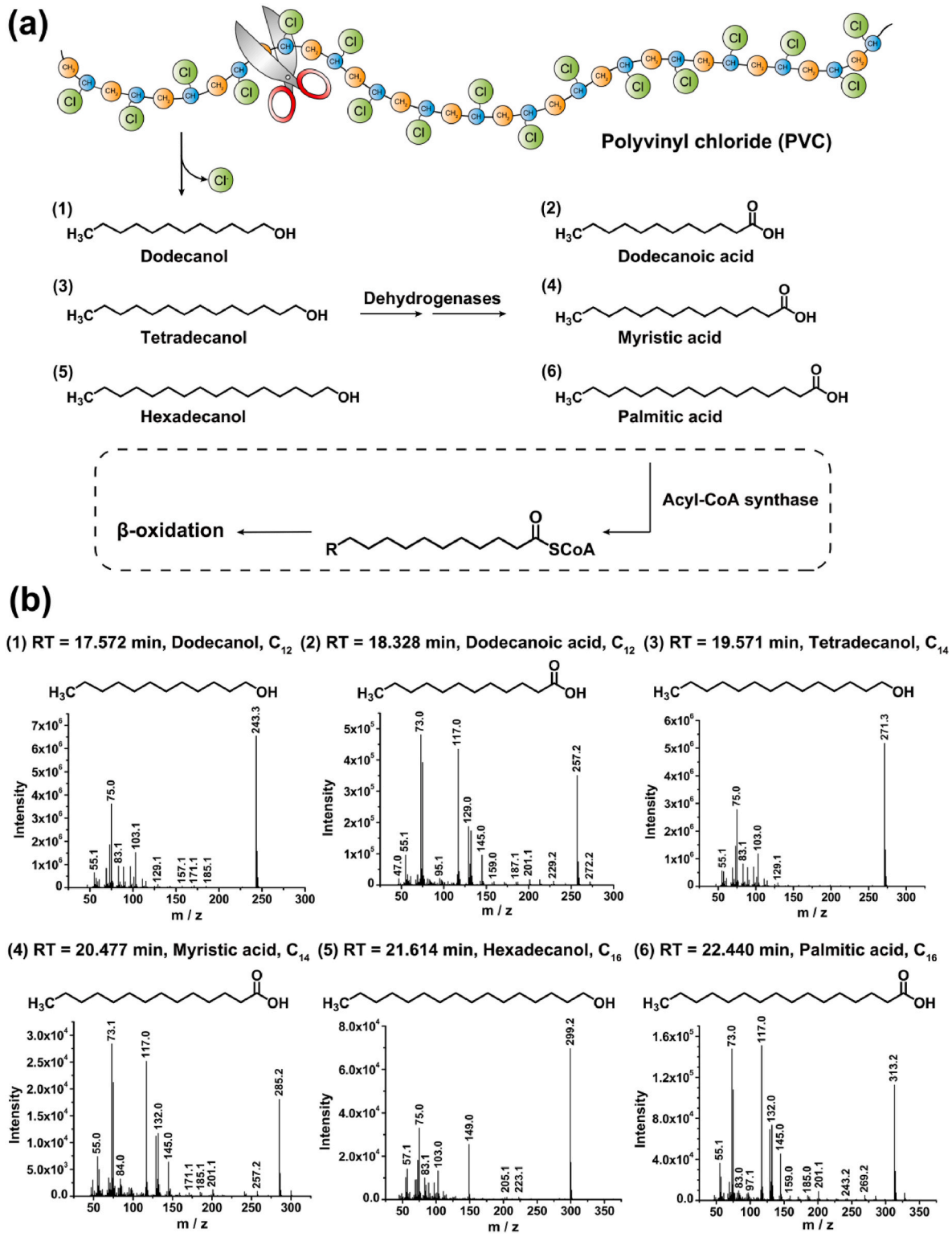

Fig. 7. Proposed degradation pathway of PVC polymer (a) and mass spectroscopy analysis of the intermediates from PVC film degradation by consortium EF1 (b). The part in the dash line box is the speculated downstream pathway during PVC degradation. A mass spectra from peaks with retention times of 17.572, 18.328, 19.571, 20.477, 21.614 and 22.440 min from GC analysis (Fig. S1) in the total ion flow were identified as dodecanol (compound 1), dodecanic acid (compound 2), tetrodecanol (compound 3), myristic acid (compound 4), hexadecanol (compound 5) and palmitic acid (compound 6), respectively.

4. Conclusion

A bacterial consortium, designated EF1, capable of degrading PVC polymer, has been successfully enriched from the gut of *Tenebrio molitor* larvae after ten-cycle enrichment in this study. According to relative abundance analysis based on V3–V4 regions of 16S rRNA gene amplicons, consortium EF1 mainly consisted of strains belonging to four phyla, ten families and ten genera. Consortium EF1 was able to utilize additive-free PVC powders as the sole carbon for its growth with dechlorination of PVC. After an incubation period of 30 d with the

consortium, 6.13% (dry weight) of PVC films was removed, but the negative control only lost less than 1%. A tight biofilm and evident cracks were observed on the bio-treated PVC film surface by SEM. "Corrosion pits" were also detected on the bio-treated PVC film surface (being thoroughly washed without extracellular secretions or biofilms) by AFM. WCA results indicated that the inoculation of consortium EF1 decreased the surface hydrophobicity and increased water-surface interaction of the PVC film. The thermodynamic analysis by TGA and DSC showed that the thermal stability of bio-treated PVC films obviously declined. Compared with those of the negative control, the reduction proportions for M_w, M_n and M_z were about 17.0%, 28.5% and 16.1% respectively by GPC. FTIR and ToF-SIMS characterization of the treatment PVC film (being thoroughly washed without extracellular secretions or biofilms) indicated that –OH and –C═C- groups increased and the chlorine content decreased. Especially, the content of OH^- increased by 37-fold, and those of $CHCl^-$, Cl^- and $^{37}Cl^-$ declined about 42.8%, 23.8% and 24.7% by ToF-SIMS, respectively. Three medium-chain aliphatic primary alcohols (C12, C14 and C16) and their corresponding medium-chain fatty acids identified by GC-MS were proposed as the intermediates of PVC degradation. All aforementioned evidences have reasonably established that consortium EF1 was able to degrade and mineralize PVC. This study has not only enriched the resource library for microbial degradation of PVC but also reasonably demonstrated cultivable microbes from guts of mealworms capable of degrading PVC, by using various analytic methods available.

Credit author statement

Ying Xu: Writing – original draft, Conceptualization, Visualization, Investigation, Funding acquisition, Project administration, Supervision, Reviewing & editing. Zhuo-Ning Xian: Writing – original draft, Visualization, Investigation. Wenlong Yue: Formal analysis, Methodology. Chao-Fan Yin: Conceptualization, Methodology. Ning-Yi Zhou: Conceptualization, Project administration, Supervision, 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.

Data availability

Data will be made available on request.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2023.137944.

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