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Biodegradation of polyvinyl chloride using vermibacteria under variable physicochemical conditions

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ABSTRACT

Plastic has a significant hazard to the environment, but its impact could be mitigated through degradation processes. Biodegradation of plastic wastes by using microorganisms is an environment-friendly method. In the current study, vermibacterial strains i.e. *Bacillus mycoides, Bacillus megaterium, Bacillus mojavensis, Bacillus thuringiensis,* and *Bacillus paranthracis,* were selected to evaluate the degrading impact on polyvinyl chloride (PVC) and tested under various physicochemical conditions such as pH (7, 5, 9), temperature (37 °C and 50 °C), carbon sources (Glucose and sucrose), and nitrogen sources (yeast extract and peptone). Liquid culture technique was used to investigate the chloride production and solid media experiment was employed for biodegradation of polyvinylchloride films. *Bacillus mojavensis* and *Bacillus paranthracis* showed maximum chloride production of 88.4 % and 87.8 % at pH 7 and 50 °C in the absence of carbon and nitrogen sources. On the other hand, *Bacillus megaterium* and *Bacillus mojavensis* showed maximum chloride production (91 % and 91.7 %) at pH 7 and 50 °C in the presence of glucose and yeast extract after 5 days of incubation while all vermibacteria indicated the highest chloride production at pH 9 and 50 °C in the presence of sucrose and yeast extract except *Bacillus thuringiensis*. Similarly, *Bacillus mojavensis, Bacillus paranthracis,* and *Bacillus thuringiensis* showed maximum chloride production in the presence of sucrose and peptone. Scanning electron microscopy was used to check the morphological changes of the PVC film after vermibacteria treatments and results revealed that vermibacteria attached to PVC films and validated the changes in surface topography. Fourier-transform infrared spectroscopy also revealed the changes in functional group intensity on both vermibacteria-treated PVC films compared to the control. It was concluded that plastic biodegradation via vermibacteria could be a potential source not only to eliminate plastic-based environmental issues but also holds the potential to significantly improve human health, reduce pollution, and support sustainable practices for a cleaner and healthier environment.

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1. Introduction

Long-chain synthetic polymeric molecules called plastics have several advantageous properties, including flexibility, strength, incredibly long durability, low weight, and low-cost production (Matjašič et al., 2021; Yogalakshmi and Singh, 2020). Although most commonly used plastics are thermoplastics such as polyethylene (PE), polystyrene (PS), polyvinyl chloride (PVC), polypropylene (PP), and polytetrafluoroethylene (PTFE) (Zeenat et al., 2021). These are non-biodegradable, accumulate in large quantities, harmful to the environment, and lead to environmental pollution (Nourollahi et al., 2019; Al-Thawadi, 2020; Yang et al., 2022; Narwal et al., 2023).

Generally, it is dumped in open areas, burned, or left as waste (landfilled, incinerated, or recycled). These methods are inefficient and unproductive because the end products of these plastic disposal methods produce harmful products which further cause damage to the environment (Sanniyasi et al., 2021) and cause health issues (Moshood et al., 2022). Various plastic degradation methods have been used in the environment such as photo-oxidative degradation, thermal degradation, ozone degradation, mechano-chemical degradation, and catalytic degradation but they pose serious health effects like carcinogenicity and mutagenicity (Gasperi et al., 2018; Wong et al., 2020). On the other hand, natural and synthetic plastics are degraded by microorganisms (actinomycetes, fungi, and bacteria) (Alshehrei, 2017). Microbial contaminants convert complex polymers into simple monomers through the natural and economical process of biodegradation (Chaudhary and Vijayakumar, 2020). Some microbial contaminants produce various kinds of intracellular and extracellular enzymes and can catalyze plastic polymers into safe smaller fragments (Agarwal and Singh, 2016). Previous literature illustrated the use of Bacillus spp. for the degradation of various types of plastics such as polyurethane (PUR) polypropylene (PP), polyethylene (PE), polyvinyl chloride (PVC), polystyrene (PS), and poly (ethylene terephthalate) (PET) (Tkachuk, 2021; Ghatge et al., 2020; Waqas et al., 2021; Restrepo-Flórez et al., 2014). They showed that *B. halodenitrificans, B. amyloliquefaciens, B. mycoides, B. brevis, B. sphericus B. circulans, B. pumilus, B. cereus, B flexus,* and *B. thuringiensis*. was shown to degrade these materials (Tkachuk, 2021). Giacomucci et al. (2019) illustrated that *B. flexus* has been able to form dense biofilm on the plastic film surface and biodegrade poly (vinyl chloride) (PVC) film.

Polyvinyl chloride (PVC) is a synthetic and thermoplastic polymer, obtained through the polymerization of vinyl chloride monomer into macromolecular chains (ComaniţĂ et al., 2016). PVC is used in appliance casings, furniture, shower curtains, toys, upholstery, and other household items, in automobile and other vehicle components, medical devices, office supplies, and packaging. However, it raises environmental concerns due to its high chlorine content and other additives, which can be harmful when PVC is disposed of in landfills or incinerated for energy recovery (Giacomucci et al., 2020). Various microbial contaminants have been used for PVC degradation such as *Pseudomonas fluorescens, Pseudomonas putida, Micrococcus luteus, Phanerochaete chrysosporium, Pseudomonas otitidis, Bacillus cereus, Acanthopleurobacter pedis, Pseudomonas aeruginosa, Aureobasidium pullulans, Rhodotorula aurantiaca, Pseudomonas citronellolis,* and *Bacillus flexus* (Giacomucci et al., 2019; Peng et al., 2020a; Luzia et al., 2020). Extensive research has been undertaken to specify microorganisms and their consortia capable of degrading PVC, including those colonizing plastic waste (Novotný et al., 2022, Ghosh et al., 2013).

In previous literature, PVC-degrading microbes were isolated from petroleum-contaminated soil or waste dumping sites (Kumar et al., 2017; Giacomucci et al., 2019). Numerous plastic-degrading microbial strains have been reported earlier i.e. *Streptococcus* sp., *Bacillus* sp., *Streptomyces* sp., *Staphylococcus* sp., *Comamonas* sp., and *Pseudomonas* sp. (Amobonye et al., 2021; Priya et al., 2022). Therefore, in the current study, vermibacterial strains (*Bacillus mycoides, Bacillus megaterium, Bacillus mojavensis, Bacillus thuringiensis,* and *Bacillus paranthracis*) were isolated from the gut of earthworms, identified as non-pathogenic and used for the first time for the PVC degradation. These vermibacteria were selected because they are not only involved in vermiremediation but also act as plant growth-promoting bacteria and are used as a microbial biofertilizer (Andleeb et al., 2022; Naseer et al., 2022) but their role as PVC degradation is still unknown. Therefore, the current study aimed to evaluate the efficacy of vermibacteria on PVC degradation under various physicochemical conditions such as pH (7, 5, 9), temperature (37 °C and 50 °C), carbon sources (glucose and sucrose), and nitrogen sources (yeast extract and peptone). It was observed that vermibacteria could restore soil health, leading to better crop yields and improved food security, which directly benefits human well-being by degrading plastic.

2. Experimental work

2.1. Chemicals, glassware, and equipment used

Laminar flow (ESCO Prod Model; EQU/03-EHC; Serial # 2000–0052), 37° incubator (MMM group Medcenter Enrich tungen GmbH), 37° shaker (Irmeco GmbH, Germany), analytical balance (SARTORIUS GMBM GOTTINGEN, Germany), centrifuge machine, electronic balance or digital weighing machine (Jeweler Precision balance Model: DH-V600A), steam sterilizer (autoclave), refrigerator, distillery plant, filter paper disc, Petri dishes, test tubes, flasks (250–1000 ml), digital camera (Cannon; Power Shot A800, USA), funnels, beakers, air tight reagent bottles, gloves, measuring cylinder, aluminum foil, stirrer, forceps, pipettes, tips, eppendorf tubes, millimeter ruler, pH meter, mineral salt media (MSM), mineral salt agar media (MSAM), polyvinyl chloride (PVC) powder, glucose, sucrose, peptone, yeast extract, tetrahydrofuran, 70 % ethanol, sodium dodecyl sulfate (SDS), dipotassium phosphate, potassium hydrogen phosphate, sodium chloride, calcium chloride dihydrate, ammonium sulphate, magnesium sulphate heptahydrate, copper sulphate, zinc sulphate heptahydrate, iron sulphate heptahydrate, boric acid, lab-lemco powder.

2.2. Collection and maintaining bacterial cultures

Five vermibacterial isolates such as *Bacillus mycoides* (OL364180), *Bacillus megaterium* (OL364178), *Bacillus mojavensis* (OL364183), *Bacillus thuringiensis* (OL364186), and *Bacillus paranthracis* (OL364187) were collected from Microbial Biotechnology Laboratory, University of Azad Jammu and Kashmir, Muzaffarabad, Pakistan and used for polyvinylchloride (PVC) degradation. All these vermibacteria were isolated from the gut of earthworm *E. fetida* (Andleeb et al., 2022; Naseer et al., 2022) possessed plant growth-promoting traits, and are non-pathogenic. Proper biosafety guidelines were used for handling vermibacteria as described by Emmert (2013). Mineral salt medium (MSM; K_2HPO_4, 0.5 g/L; KH_2PO_4, 0.04 g/L; NaCl, 0.1 g/L; $CaCl_2.2H_2O$, 0.002 g/L; $(NH_4)_2SO_4$, 0.2 g/L; $MgSO_4.7H_2O$, 0.02 g/L; $CuSO_4$, 0.001 g/L; $ZnSO_4.7H_2O$, 0.01 g/L; $FeSO_4.7H_2O$, 0.001 g/L; Boric acid, 0.005 g/L; Agar in case of solid media; MSAM) was used for degradation studies, and nutrient medium (NM; Lab-lemco powder, 1 g/L; Yeast extract, 2 g/L; Sodium chloride, 5 g/L; Peptone, 5 g/L; Agar in case of solid media) was used for maintaining bacterial cultures. Vermibacteria were grown in a nutrient broth medium for 16 h at 37 °C and further used for PVC degradation.

2.3. Polyvinyl chloride (PVC) film formation

Polyvinylchloride film was prepared by solvent casting method in which 1 % PVC powder was dissolved in tetrahydrofuran (Nishida and Tokiwa, 1992). After dissolving, the solution containing tetrahydrofuran and PVC was poured into a clean Petri plate to make a PVC film. The Petri plate was left overnight at room temperature (25 °C.±2.0). After 16 h of incubation, tetrahydrofuran was evaporated, and the film was picked from the Petri plate with a sterilized spatula spoon. PVC film was

kept at room temperature for overnight further (Fig. 1. A). The next day, PVC films were sterilized by dipping them into 70 % ethanol for 20 min, washed with sterilized water, air-dried, and cut into small strips (30×30 mm) (Calil et al., 2006).

2.4. Polyvinyl chloride degradation experimental design

Two different methods i.e., conical flask (broth) and solid agar methods were used for screening of PVC degradation using vermibacteria (Fig. 1. B). Mineral salt media was used for both methods to check the efficacy of PVC degradation by vermibacteria at various physicochemical parameters i.e., pH (5, 7, and 9), temperature (37 °C and 50 °C), carbon (glucose and sucrose) and nitrogen sources (yeast extract and peptone) respectively. These physicochemical parameters were selected due to their significant effects on the biodegradation of plastics using vermibacteria. The biodegradation process may work faster or slower depending on the pH levels, temperature as well as nutrient availability. The specific microbial activities depend upon the presence or absence of certain nutrients especially carbon and nitrogen sources, which not only enhance the growth of microbes but also speed up the degradation process.

2.4.1. Conical flask method

For the conical flask experiment, the methodology was used with slight modifications as described by Kumar et al. (2017). Approximately one gram of PVC film was transferred in the conical flasks containing 50 mL of MSM along with 400 µL of different overnight vermibacterial cultures. The pH of the media was adjusted and sterilized. Inoculated flasks were placed at various temperatures for consecutive 5 days at 180 rpm. Two controls were kept for the current study: 1. PVC film and MSM in the bacteria-free medium, and 2. MSM containing flask without PVC and bacterial culture. After 24 h, 48 h, 72 h, and 120 h of incubation, 10 mL of sample was taken from each flask for the analysis of released chloride concentration as prescribed by Yabannvar and Bartha (1994). The collected samples were centrifuged at 4500 rpm for 15 min and supernatant was collected for chloride concentration measurement. Chloride concentration was determined by spectrometric analysis (Bergmann and Sanik, 1957) at 650 nm.

2.4.2. Solid media experiment

In the solid media experiment, the pour plate technique was used with slight modifications (Cornell et al., 1984). Mineral salt agar medium was prepared, pH (5, 7, and 9) of the media was adjusted and sterilized. The overnight bacterial culture was mixed with prepared MSAM at 45 °C, poured into Petri plates, and placed at room temperature for solidification in laminar flow. After solidification, the small strips of PVC films were placed on the surface of the medium, incubated at 37 °C and 50 °C for 28–30 days, and observed the growth of bacterial strains around PVC films. Two controls were kept for the study: 1. PVC film + MSAM in the bacteria-free medium, MSAM containing petri plate without PVC, and bacterial culture. Further PVC film was collected, washed, dried, and sealed in plastic bags for SEM and FTIR analysis. After the in vitro experiment, an ex-situ experiment was carried out to evaluate the PVC degradation effect of vermibacteria (*Bacillus megaterium, Bacillus mojavensis, Bacillus thuringiensis,* and *Bacillus paranthracis*) in natural soil as involved in maximum PVC degradation. After 30 days of incubation, PVC films were analyzed through SEM. **B1**: *B. mycoides* **B2:** *B. megaterium* **U2:** *B. mojavensis* **U5:** *B. thuringiensis*, **U6***: B. paranthracis*

2.5. Fourier transform infrared (FTIR) spectroscopy

FTIR was used to detect the formation and disappearance of functional groups in the PVC polymer during the degradation process. Vermibacteria-treated PVC films and controlled PVC films were mixed with KBr and fixed with a sample holder. A spectrum was taken at 400–4000 cm^{-1} for each sample in triplicates was monitored.

2.6. Scanning electron microscopy (SEM)

The surface morphology of vermibacteria-treated PVC films and controlled PVC films were analyzed through Scanning Electron Microscopy and compared.

2.7. Statistical analysis

All treatments were conducted in triplicates and the results were presented as a mean value with standard deviation (Mean ± SD). Mean and standard deviation were calculated using online software https://www.calculator.net/standard-deviation-calculator.html. The statistical significance was assessed by one-way analysis of variance (ANOVA). *represents a significant change among treated groups at $p \leq 0.01$. The chloride production difference between treated PVC and control is calculated as (Absorbance of test − Absorbance of control) x 100.

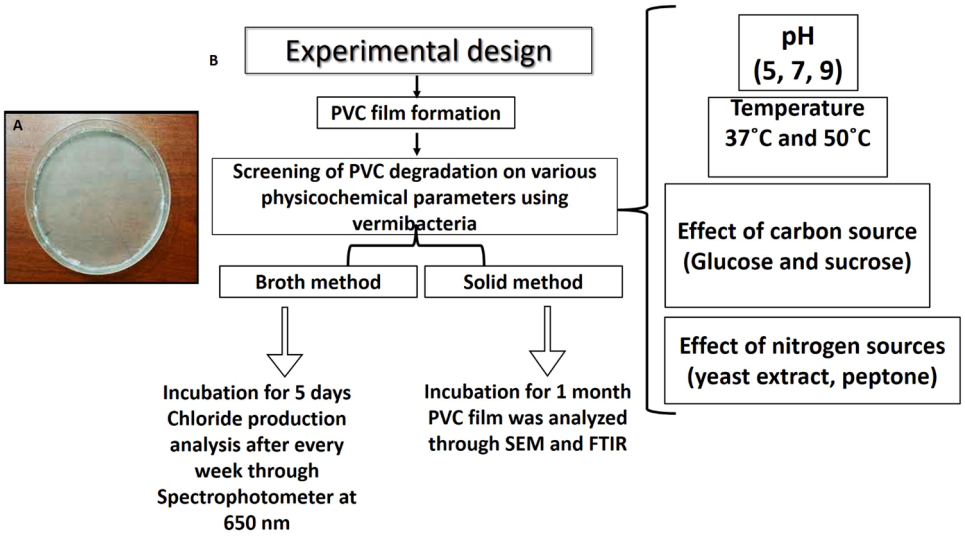

Fig. 1. Screening of PVC degrading vermibacteria on various physicochemical parameters.

3. Results and discussion

Biodegradation can be characterized by loss of weight, change in tensile strength, change in dimensions, change in chemical and physical properties, carbon dioxide production, bacterial activity in the soil, and change in molecular weight distribution (Sivan, 2011; Kumar and Maiti, 2016; Chen et al., 2020a; 2020b). Similarly, several methods have been developed by American Society for Testing and Materials (ASTM) and International Standard Organization (ISO) including micro-Fourier transform infrared spectroscopy (m-FTIR), gas chromatography/-mass spectrometry (GC–MS), and stereomicroscopy (Piergiovanni and Limbo, 2016; Lomonaco et al., 2020; Corami et al., 2020) to assess the biodegrading ability of plastics. Similarly, changes in the PVC film texture were checked via scanning electron microscopy, and FTIR analysis was used to detect the formation of new functional groups or changes in the amount of existing functional groups (Nyamjav et al., 2023). In the current study, PVC degradation by vermibacterial strains was determined by using the method based on chloride release as previously used by Yabannvar and Bartha (1994). The morphology changes in vermibacteria-treated PVC were analyzed through FTIR and SEM, respectively.

3.1. Chloride production via the shake flask method at various temperatures and pH in the absence of carbon and nitrogen sources

Physicochemical parameters (pH, temperature, carbon, and nitrogen source) are essential for the growth of bacteria and currently utilized for the biodegradation of PVC by using *Bacillus spp* (Peng et al., 2020a; Miloloža et al., 2022; Novotný et al., 2022). In the current research, PVC film was treated with various vermibacterial isolates (*Bacillus spp)* at different temperatures (37 °C and 50 °C) and pH (5, 7, 9) to evaluate the PVC degradation effect *via* chloride production (Fig. 1; Supplementary Table 1). Results indicated that *B. mojavensis* showed the highest and most significant ($p \leq 0.01$) chloride production (0.79±0.0: 77.7 %) after 120 h while *B. megaterium* showed 0.449±0.0 chloride production after 72 h of incubation at 37 °C and 5 pH (Fig. 1; Supplementary Table 1). On the other hand, at pH 7, *B. paranthracis* showed the highest chloride production 0.202±0.149 after 120 h while *B. thuringiensis* produced 0.193±0.0 chloride after 96 h of incubation (Fig. 1; Supplementary Table 1). Similarly, at pH 9, *B. megaterium* showed 0.333±0.001 chloride production after 120 h in the absence of carbon and nitrogen sources (Fig. 1; Supplementary Table 1). Overall findings indicated that *B. mojavensis*) vermibacteria showed 77.7 % chloride production at pH 5 among all tested vermibacterial isolates in the absence of carbon and

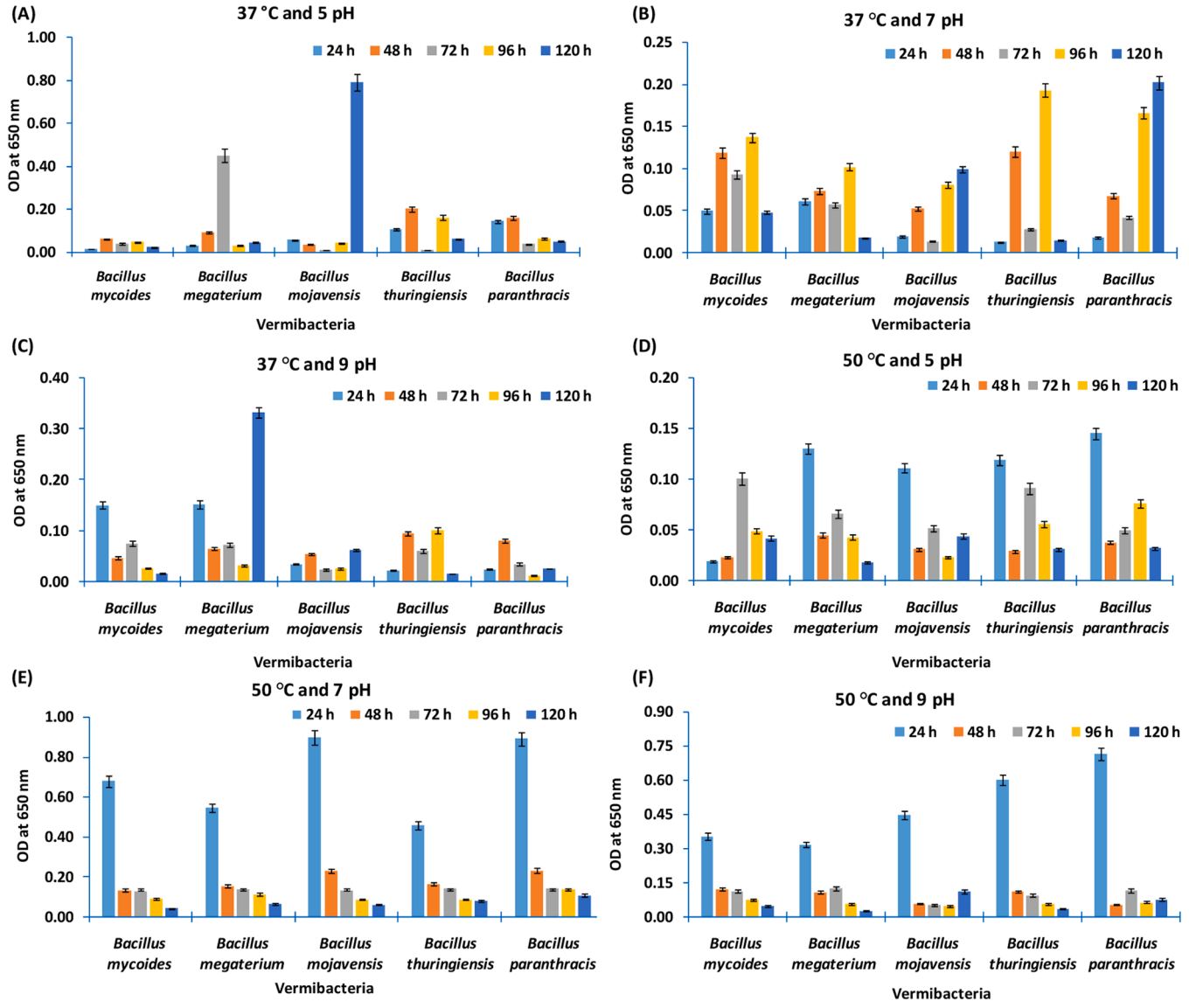

Fig. 2. (A-F): Chloride production *via* vermibacterial isolates at various pH and temperature in a minimal medium without carbon and nitrogen source at 650 nm.

nitrogen sources (Fig. 2). Interesting results were recorded when PVC film was treated with various vermibacterial isolates at 50 °C and different pH. Results revealed that all vermibacteria showed significant chloride production at 7 pH and 9 pH after 24 h compared to pH 5 at ($p \leq 0.01$). It was also observed that the chloride production gradually decreased after each 24 h of incubation at 50 °C (Fig. 2; Supplementary Table 2). The current findings agreed with Oda et al. (2018). This study reported that pH and temperature play a crucial role in the biodegradation of plastics as well as in the growth and development of microbes. The degradation of plastics increased to >30 % with increased temperatures (55 °C, 60 °C, and 65 °C), similar to current outcomes. The findings of the current study also agreed with the outcomes of Al-Salem et al. (2019), who illustrated that temperature plays an important role in plastic degradation. It was observed that the degradation process and chloride production in the flask method also depend on the pH, temperature, and incubation period. In the current results, the degradation process/chloride production is increased with an increase in the physical parameters, and increased pH enhances the degradation process.

3.2. PVC film degradation via solid media

The shake flask results revealed the maximum degradation of PVC at 50 °C via measuring chloride production. Based on these findings, PVC film degradation via the solid method was conducted at 50 °C using various pH levels (5, 7, and 9) in the absence of carbon and nitrogen sources compared to 37 °C. The results indicated that most vermibacterial isolates exhibited maximum growth on and around the surface of the PVC film at pH 7 and 9 after incubation of 20–28 days (Fig. 3). Findings of this study align with the previous literature (Giacomucci et al., 2019; Kumari et al., 2019, 2020). Giacomucci et al. (2019) used various bacterial contaminants such as *Pseudomonas citronellolis, Pseudomonas chlororaphis, Bacillus subtilis, Chelatococcus daeguensis,* and *Bacillus flexus* for the degradation of polystyrene polypropylene, polyethylene, and polyvinyl chloride films under aerobic conditions and showed that PVC reduced weight after 30 days of incubation. Kumari et al. (2019) also reported the PVC, LDPE, and HDPE degradation using marine ecosystem-associated *Bacillus sp*. Similarly, the current results are consistent with the outcomes of Zeenat et al. (2021), who demonstrated that the microbial biodegradation of polymers consists of three steps; A) microbial attachment to the polymer's surface; B) using the polymer as a carbon source; and C) degradation of polymer. It can be attributed that all vermibacteria can attach to PVC film, use PVC as a carbon source, and depolymerize PVC films. PVC degradation was carried out by the releasing of microbial enzymes which agreed with the study of Danso et al. (2018). The current findings are also consistent with the outcomes of Brandon et al. (2018) and Kundungal et al. (2019). Peng et al. (2020b) and Yang et al. (2022) reported that plastic depolymerization is dependent on the gut microbe of *Zophobas atratus* and *Tenebrio molitor* larvae. Literature also reported that gut microbial contaminants of the insects has been used for PE and PS degradation (Wang et al., 2020; Kumar et al., 2022) but more evidence is needed to be provided.

The current study proved that invertebrates have a significant role in the plastic degradation and findings agreed with previous literature as they verified the plastic degradation via other invertebrates such as lesser waxworm (Kundungal et al., 2019), flour beetle (Kundungal et al., 2019), land snail (Song et al., 2020), anecic and endogeic species of earthworms (Sanchez-Hernandez et al., 2020; Christyraj et al., 2022; Wang L. et al., 2022).

3.3. Chloride production via shake flask method under various pH in the presence of carbon and nitrogen sources at 50 °C

3.3.1. Glucose, sucrose, and yeast extract

Based on findings of PVC degradation of solid media at 50 °C and various pH 7 and 9 after 24 h, further experiments were carried out to evaluate the PVC degradation *via* chloride production in a shake flask experiment at 50 °C for 120 h at different pH (7 and 9) under various combinations of carbon (glucose and sucrose) and nitrogen (peptone and yeast extract) sources i.e. Glucose+Yeast extract; Glucose+Peptone; Sucrose+Yeast extract; and sucrose+peptone, respectively. The significant ($p \leq 0.01$) increased chloride production with an increased

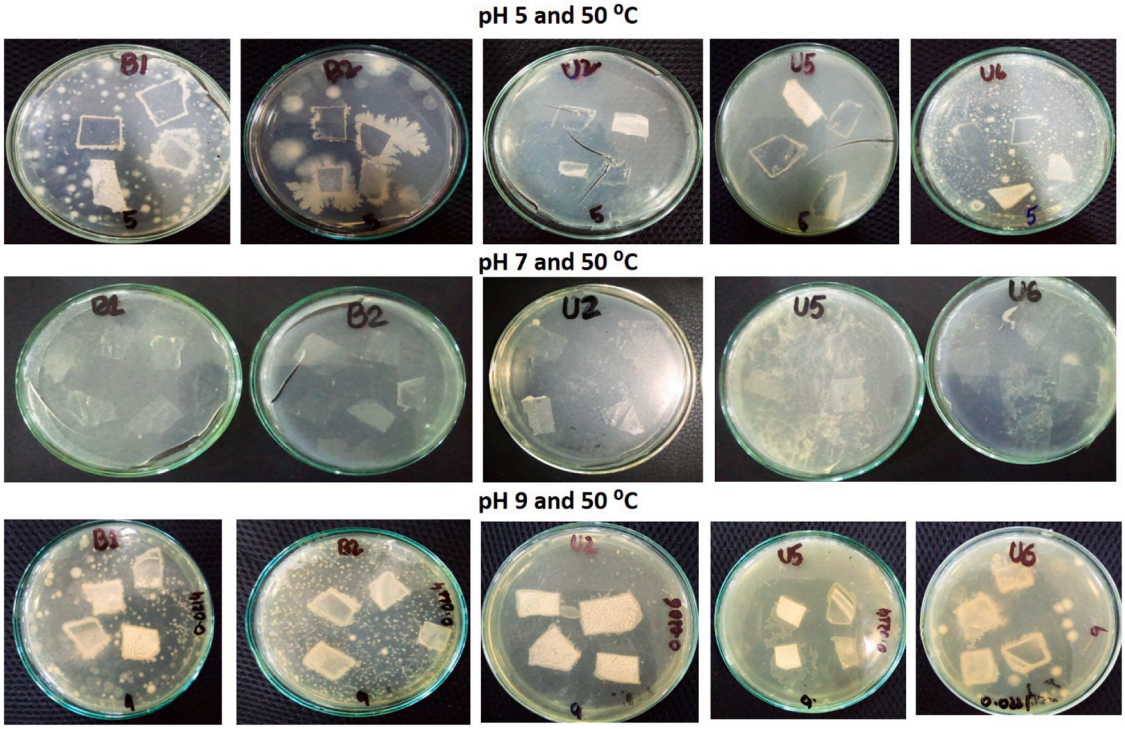

Fig. 3. PVC film degradation using various vermibacterial isolates at 50 °C and different pH i.e. 5, 7, and 9 without carbon and nitrogen sources. B1: *B. mycoides,* B2: *B. megaterium,* U2: *B. mojavensis,* U5: *B. thuringiensis,* U6*: B. paranthracis.*

incubation period in the presence of carbon and nitrogen sources as well as on both tested pH values (7 and 9) and maximum PVC degradation was also recorded (Supplementary Tables 3 and 4). *B. megaterium* showed chloride production (0.952±0.0; 60.2 % and 1.226±0.0; 87.6 %) after 48 h of incubation at 7 and 9 pH in the presence of sucrose and yeast extract (Supplementary Table 3; Fig. 4). On the other hand, *B. mycoides* showed 1.02±0.0 chloride production (100 %) at 9 pH after 48 h of incubation, followed by 93 % at 7 pH after 48 h incubation. *B. mojavensis* showed 91.7 % chloride production at 9 pH after 48 h of incubation. Findings showed significant chloride production via *B. megaterium, B. mycoides*, and *B. mojavensis* at ($p \leq 0.01$).

3.3.2. Glucose, sucrose, and peptone

B. mojavensis produced 0.67±0.003; 62.4 % chloride at 7 pH in the presence of glucose+peptone after 48 h of incubation while *B. megaterium* and *B. paranthracis* showed 0.704±0.004;63.7 % and 0.678±0.002;60 % chloride production at 9 pH after 48 h of incubation (Supplementary Table 4; Fig. 4). Interesting results were recorded when sucrose was used in combination with peptone. *B. mojavensis* and *B. thuringiensis* showed the maximum chloride production (1.923 ±0.022; 96.2 % and 1.998±0.017; 67.7 %) at 7 pH in the presence of sucrose+peptone after 48 h of incubation. On the other hand, *B. megaterium, B. thuringiensis, B. paranthracis* increased chloride production (1.23±0.002; 66.2 %, 2.231±0.019; 91 %, and 2.201±0.002; 96.7 %) at 9 pH after 48 h of incubation (Supplementary table 4; Fig. 4). Overall, the findings revealed that *B. thuringiensis* and *B. paranthracis* showed significant chloride production (91 % and 96.7 %) in the presence of sucrose and peptone at pH 9 and 50 °C compared to a medium having no carbon and nitrogen source at ($p \leq 0.01$). The reason might be that microbial growth was more robust at this pH level with the nutritive

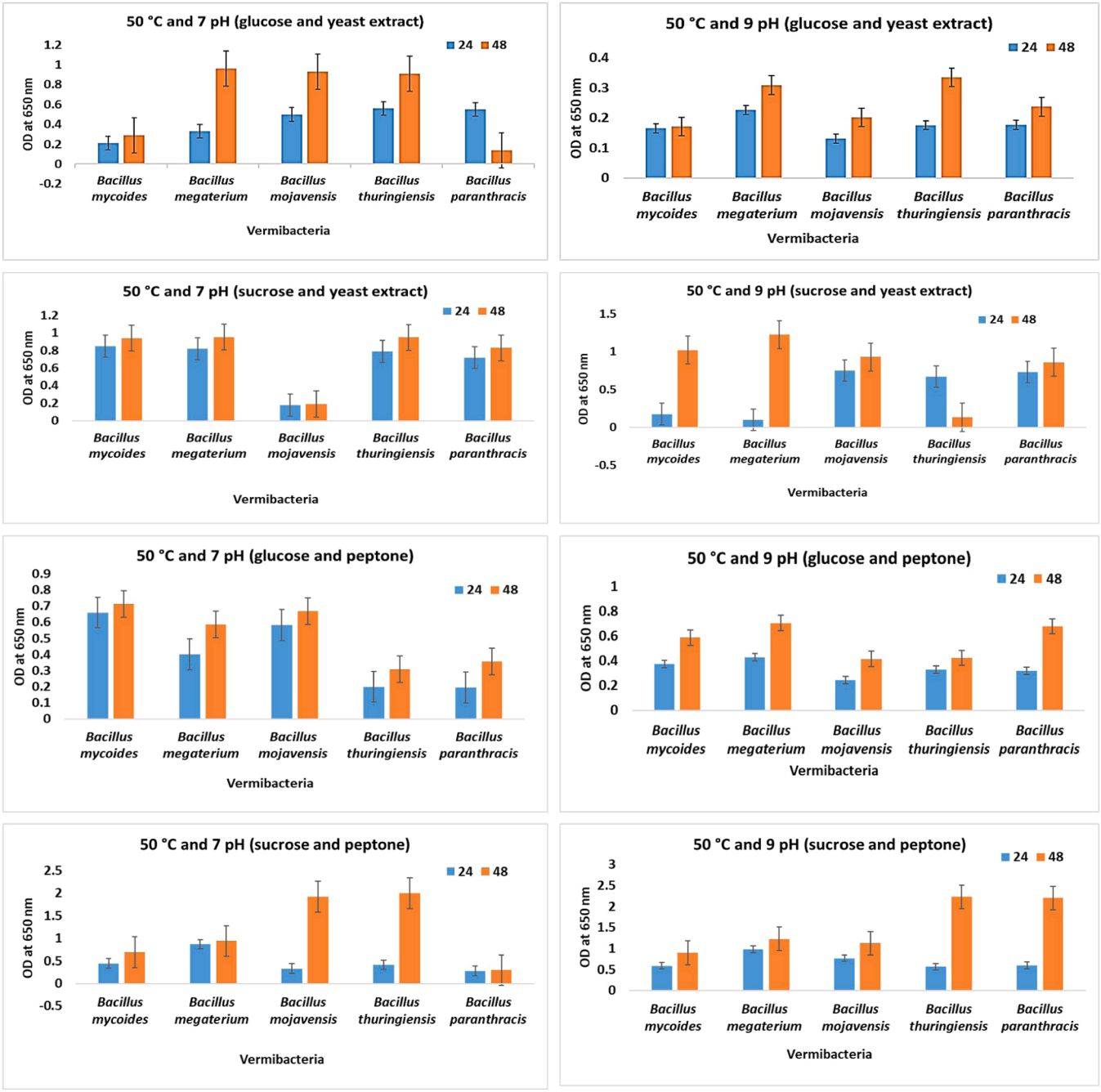

Fig. 4. Chloride production *via* vermibacterial isolates at various pH and 50 °C in a minimal medium with combinations of carbon and nitrogen sources at 650 nm.

medium compared to the other pH without the nutritive medium, leading to a higher degradation rate, which aligns with the findings of Saeed et al. (2022). The current research results agreed with Sarkhel et al. (2020), who evaluated the pH at 1, 3, 7, 9, and 11, and it was discovered that as the pH increased from pH 1 to pH 11, the degradation rate of the polymer increased, with the best results obtained at alkaline pH. On the other hand, carbon and nitrogen sources also enhanced the degradation process as well as chloride production. Similarly, the highest chloride production occurred in the presence of sucrose and peptone showing a greater rate of degradation. According to Saeed et al. (2022), PVC film lost weight at 37 °C which indicated better degradation. Similarly, Sarkhel et al. (2020) also examined the effect of temperature on the rate of plastic degradation between 25 °C and 45 °C. They observed a greater rate of degradation at 35 °C. On the other hand, in the current study, PVC degradation was observed at 37 °C and 50 °C, and the maximum PVC degradation was observed at 50 °C temperature. Results revealed that sucrose and peptone were the best medium composition for PVC degradation using vermibacteria.

3.4. *PVC film degradation at various pH CONDITIONS, carbon and nitrogen sources at 50 °C via agar method*

Results revealed that PVC film was degraded via vermibacterial isolates in the presence of carbon and nitrogen sources at 50 °C using various pH (7, and 9). On the solid medium, the maximum growth of vermibacterial isolates on and around the surface of PVC film was recorded in the combination of glucose, yeast extract, sucrose, and peptone at 50 °C and 9 pH (Figs. 5 and 6). Similarly, the maximum growth of *B. mojavensis, B. thuringiensis,* and *B. paranthracis* were recorded on and around the PVC film at 50 °C and both 7 and 9 pH.

3.5. *SEM analysis*

SEM analysis reveals the significant changes in the physical structure of the PVC film when treated with vermibacterial isolates in both in vitro and ex-situ analysis. After treatment, multiple holes/cracks and changes in the surface structure of the plastic film were observed to aid in the biodegradation of these plastics compared to the control PVC film. Before being treated with microbial isolates, the surface of polymer films was smooth and shiny. The smoothness of the PVC film surface was lost after the degradation assay, as confirmed by SEM analysis. Variations such as erosion, pits, and cracks were observed on the polymer's surface area as a result of bacterial cell attachment via biofilm formation (Fig. 7. I. and 7.II.). Scanning Electron Micrographs of the treated and untreated PVC films showed marked changes in surface morphology as well as microbial attachment. The roughening of the surfaces as well as the formation of grooves, pits, and cracks showed the ability of microbial consortia to degrade the samples in solid media. The current research results agreed with the outcomes of Han et al. (2020). They observed the surface morphological and functional group changes, reduced weight,

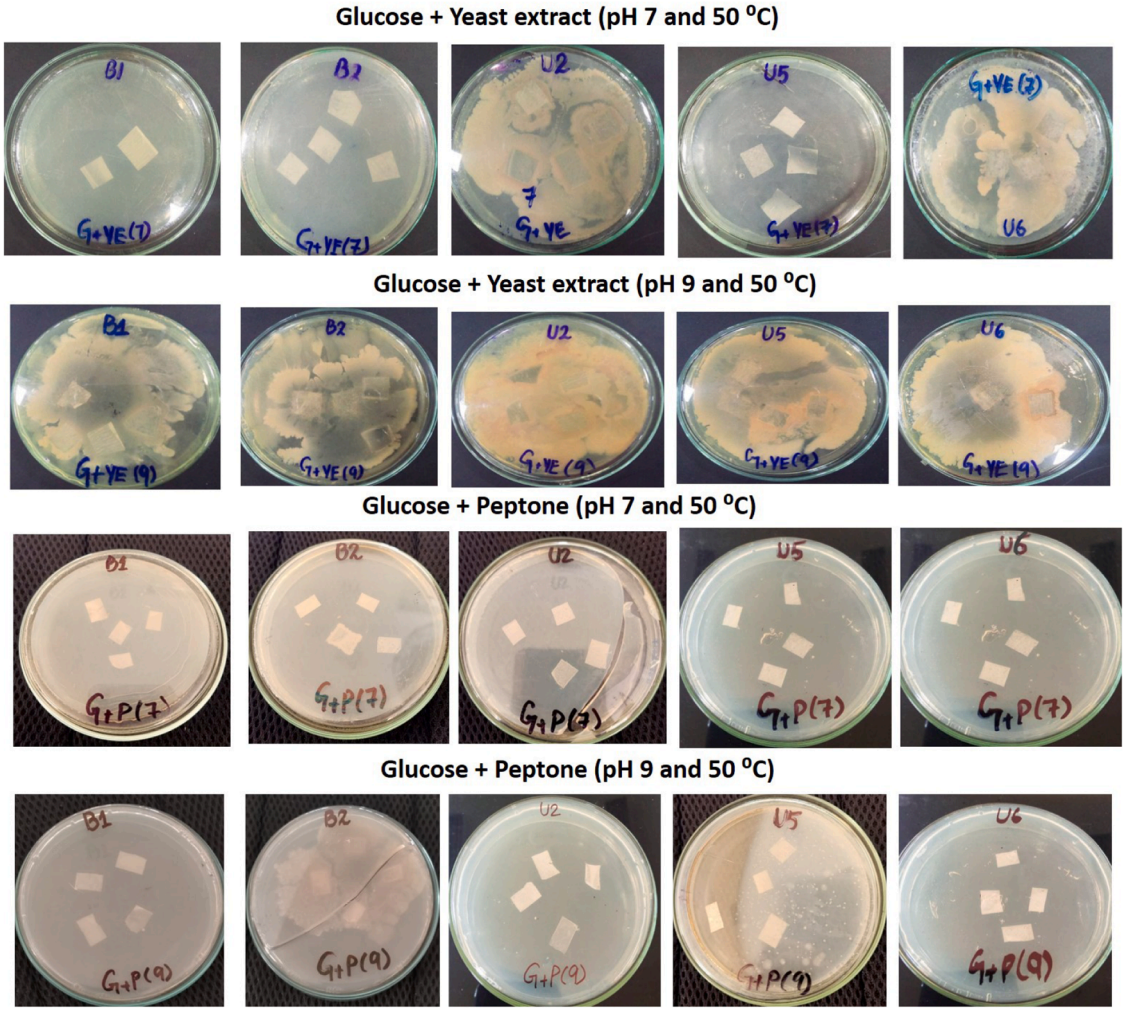

Fig. 5. PVC film degradation using vermibacterial isolates in the presence of glucose, peptone, and yeast extract at 50 °C and various pH. B1: *B. mycoides* B2: *B. megaterium* U2: *B. mojavensis* U5: *B. thuringiensis* U6*: B. paranthracis*.

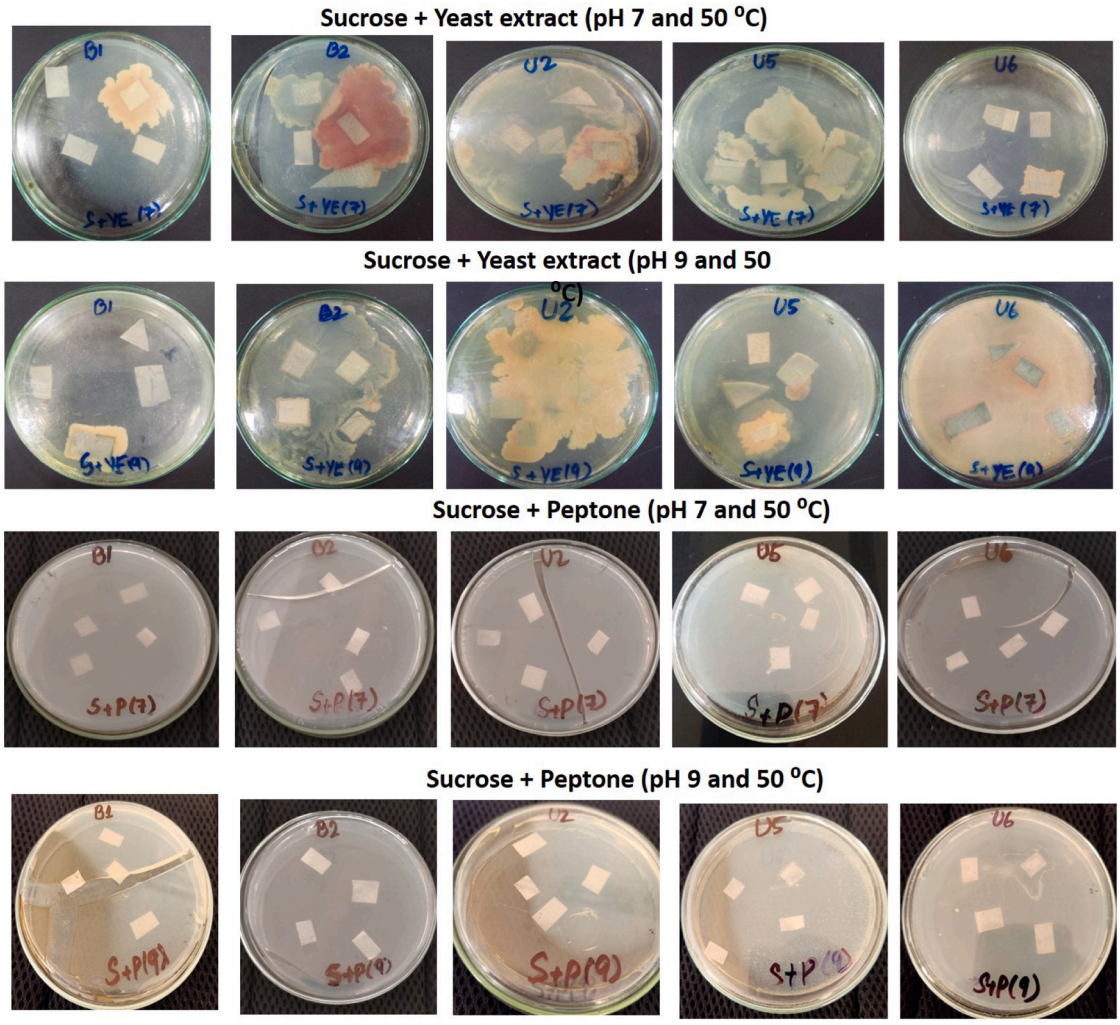

Fig. 6. PVC film degradation using vermibacterial isolates in the presence of sucrose, peptone, and yeast extract at 50 °C and various pH. B1: *B. mycoides* B2: *B. megaterium* U2: *B. mojavensis* U5: *B.* thuringiensis: U6*: B. paranthracis*.

and hydrophobicity of polymer when biofilm is formed on its surface. Biofilm initiation enhances the carbon utilization by microbes. Scanning electron microscopy of PVC films at the end of incubation showed many changes in surface morphology, such as erosion and extensive roughening of the surface with pit formation compared to untreated PVC films (Fig. 7). Multiple studies suggested that variance may be caused by the action of microbial extracellular or intercellular enzymatic actions (Skariyachan et al., 2016, 2018, 2021; Amobonye et al., 2021). According to Bhatia et al. (2014), bacterial and fungal enzymatic reactions are most likely to be responsible for the surface erosion process involved in the degradation of plastics. The extracellular enzymes are released into the medium and involved in the polymer hydrolysis (Wilkes and Aristilde, 2017). The current results are consistent with Wilkes and Aristilde (2017). Various enzymes are produced by these vermibacterial isolates such as proteases, lipases, esterases, oxidases, and amylases, which could be involved the PVC degradation. The current study revealed that these enzymes are involved in the multistep process of degradation (1. Depolymerization, 2. Fragmentation/mineralization, and 3. Assimilation) and agreed with Bhal et al. (2021) and Chaurasia (2020). Magnin et al. (2020) illustrated that Cutinases, lipases, proteases, and ureases released from *Aspergillus niger* and *Chaetomium globosum*, and involved in the polyurethanes degradation.

3.6. Fourier transform infrared spectroscopy

PVC-non-treated films (control) showed spectral peaks corresponding to C—H stretching, C—H bending, and C—O-C bonding (Table 1). On the other hand, FTIR results of PVC-treated films in the presence of sucrose and peptone reveal that different vermibacteria have diverse PVC degradation mechanisms such as bio-deterioration, bio-fragmentation, bio-assimilation, and bio-mineralization (Montazer et al., 2020; Bahl et al., 2021). The PVC degradation indicates several changes or shifts in the peaks of PVC functional groups which may be due to various physicochemical parameters used for the treatment processes (Table 1; Fig. 8). The characteristic peaks seen in vermibacteria PVC-treated films are shown in Table 1. These peaks corresponded to the–CH2– deformation, C—H stretching (3000–2800 cm^{-1}), C–Cl stretch vibration, C—H bending and stretching, C–H and N–H deformation vibrations, C—O-C bonding, stretch vibration of the carbonyl group (C = O), and C—O bonds. Similarly, PVC-treated films revealed spectra that corresponded to C—H stretching, C—H bending, C—O-C bonding, and degradation of C—O bonds (1300–800 cm^{-1}). Furthermore, FTIR analysis validates the enzymatic action of vermibacteria in PVC degradation, weight loss, and removal of molecules which was in keeping with other reports (Giacomucci et al., 2019; Ru et al., 2020).

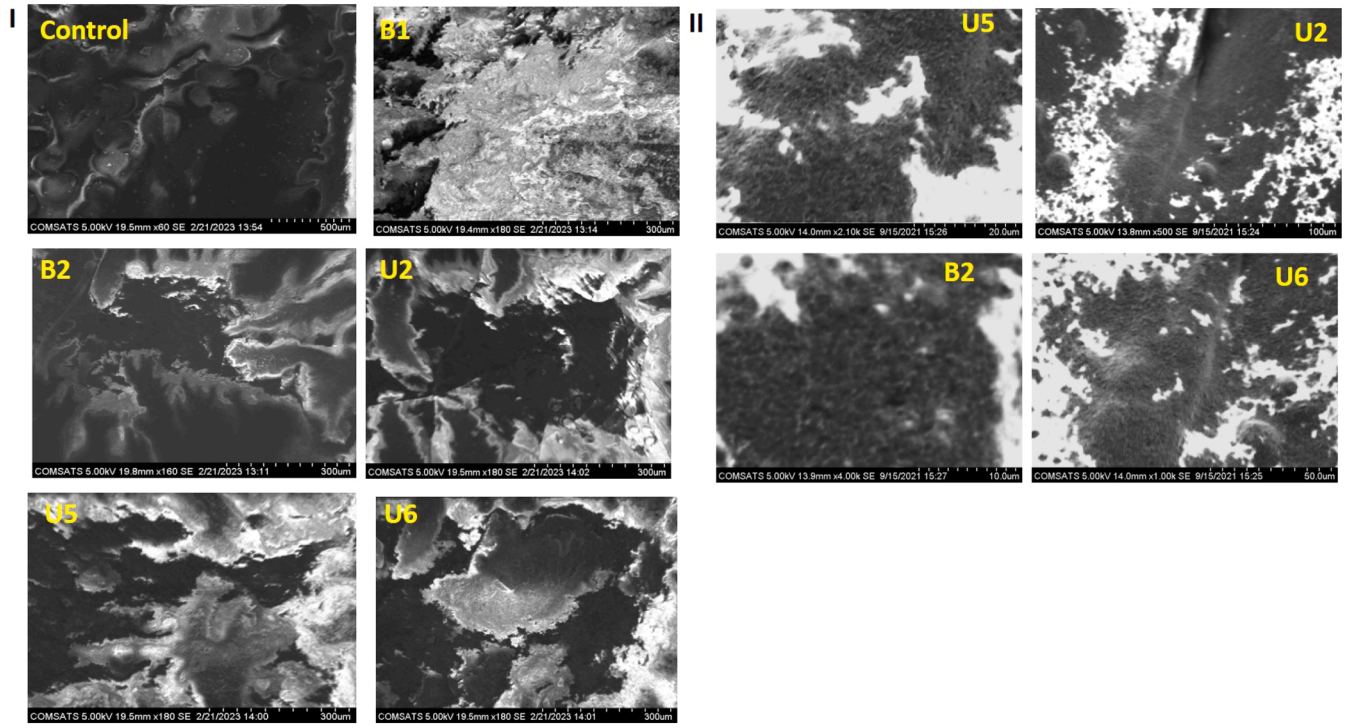

Fig. 7. SEM analysis of PVC film treated with vermibacterial isolates. I. PVC degradation on agar medium via vermibacteria; **II.** Ex-situ PVC degradation in soil having vermibacteria; **B1**: *B. mycoides* **B2:** *B. megaterium* **U2:** *B. mojavensis* **U5:** *B. thuringiensis* **U6***: B. paranthracis*.

Table 1
FTIR analysis of Vermibacteria PVC-treated films.

Peaks cm^{-1}					
PVC-non treated film	PVC-treated films				
Control	B. mycoides	B. megaterium	B. mojavensis	B. thuringiensis	B. paranthracis
2965.06, 2910.22, 2852.75, 1425.33, 1378.54, 1327.02, 1245.59, 1090.60, 958.47, 867.02, 835.30, 687.74	2963.86, 2908.83, 2849.06, 1424.21, 1323.65, 1238.52, 1199.17, 1086.22, 1055.01, 1026.39, 956.47, 833.70, 683.21, 607.11 and 492.47 cm^{-1}	2966.18, 2908.83, 1426.56, 1327.46, 1245.35, 1201.96, 1092.64, 958.12, 687.02 and 611.00 cm^{-1}	2957.97, 2907.62, 2843.17, 1594.22, 1467.04, 1422.58, 1323.57, 1236.26, 1196.44, 1090.11, 1063.37, 955.50, 922.98, 872.93, 834.15, 678.92, 606.19, 521.71, 487.5	2904.99, 2221.86, 2186.52, 2140.66, 2054.90, 2023.94, 1993.70, 1424.03, 1321.45, 1232.50, 1092.53, 1061.61, 1032.67, 1008.35, 954.02, 928.56, 891.50, 865.75, 819.47, 781.13, 750.34, 684.07, 602.89, 539.87, 516.70 cm^{-1}	2963.23, 2909.84, 2850.55, 1426.46, 1327.18, 1245.51, 1094.15, 959.38, 678.08, 612.29

4. Conclusions and future recommendations

The biodegradation of PVC through vermibacteria associated with the gut of *Eisenia fetida* presents an innovative solution to address plastic pollution. In this study, PVC degradation using vermibacteria was investigated in both in-situ and ex-situ environments under varying physicochemical conditions. The results confirmed that vermibacteria from the gut of *E. fetida* could effectively degrade PVC plastic when carbon and nitrogen sources were present. Evidence from chloride production, scanning electron microscopy (SEM), and Fourier-transform infrared (FTIR) analysis further supported this degradation process. The most efficient PVC breakdown occurred in media containing sucrose and peptone. The effectiveness of PVC degradation by bacteria was influenced by several factors, including physical parameters, bacterial load in the medium, bacterial growth and attachment to the PVC film, and the composition of the medium. Findings proved that vermibacteria show promise in PVC (polyvinyl chloride) degradation due to their unique enzymatic capabilities and symbiotic relationships with earthworms, which may facilitate enhanced breakdown of this resilient plastic. Unlike other microbes, vermibacteria are part of a specialized digestive environment within earthworms that can process complex and recalcitrant materials, including PVC, through a combination of physical breakdown and microbial digestion. Their enzymes, particularly those capable of cleaving carbon-chlorine bonds, are effective in attacking PVC's tough polymer structure, a task that many microbes struggle to accomplish due to the plastic's high resistance to degradation. Additionally, the presence of earthworms as hosts provides an optimal, moist, and nutrient-rich microenvironment that supports vermibacteria activity, further differentiating them from other microbes which may lack the ecological or enzymatic adaptations needed for efficient PVC degradation. Further research is required to explore the use of vermibacteria as a plastic degrader in ex-situ environments, better understand the PVC degradation mechanism, and assess the bacteria's stability and potential to degrade other plastic materials. Vermibacteria, despite their potential for plastic degradation, face several limitations. These bacteria often have specific environmental requirements, such as optimal temperature, pH, and nutrient availability, which can restrict their large-scale application. Additionally, their degradation rate is relatively slow, limiting the efficiency in breaking down plastics in practical, commercial settings. There are also challenges in ensuring they target plastics without affecting other organic materials in the environment. Lastly, producing and maintaining vermibacteria cultures on an industrial scale is costly

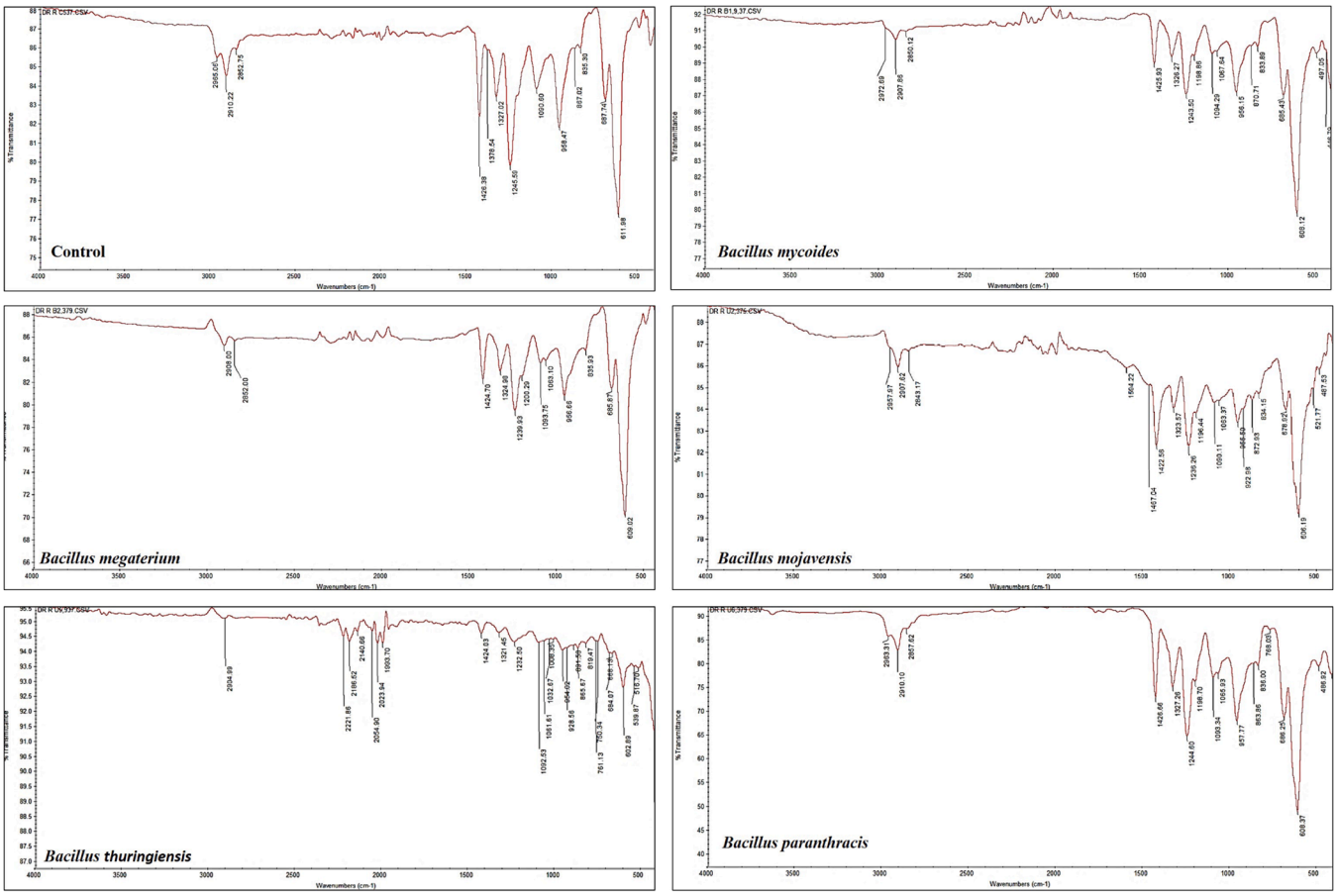

Fig. 8. FTIR spectra of PVC-treated films with vermibacterial isolates under sucrose and peptone.

and can have ecological implications, particularly if they interact with native ecosystems in unintended ways.

CRediT authorship contribution statement

Saiqa Andleeb: Writing – review & editing, Writing – original draft, Validation, Project administration, Investigation, Data curation, Conceptualization. **Muqaddas Munir:** Writing – review & editing, Validation, Formal analysis, Data curation. **Muhammad Ishtiaq Ali:** Writing – review & editing, Validation, Formal analysis. **Kaleem Imdad:** Writing – review & editing, Writing – original draft, Validation, Data curation. **Ramalingam Balachandar:** Writing – review & editing, Investigation. **Ravishankar Ram Mani:** Writing – review & editing, Writing – original draft, Validation, Project administration, Funding acquisition, Conceptualization. **Murugesan Chandrasekaran:** Writing – review & editing, Validation, Investigation. **Sumathi Jones:** Writing – review & editing, Supervision, Investigation. **Arunkumar Radhakrishnan:** Writing – review & editing, Validation, Investigation. **Soon Woong Chang:** Writing – review & editing, Validation, Investigation. **Balasubramani Ravindran:** Writing – review & editing, Writing – original draft, Project administration, Investigation, Data curation, Conceptualization.

Declaration of competing interest

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

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.hazadv.2024.100571.

Data availability

Data will be made available on request.

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