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Isolation, characterization and effect of bioformulation on bacterial isolate derived from e-waste enriched soil

Moumita Chakraborty, J. P. N. Rai, Ankita Ghosh, Diksha Sah

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Authors:
M. Chakraborty ✉, J. P. N. Rai, A. Ghosh, D. Sah
Department of Environmental Sciences, College of Basic Sciences & Humanities, G. B. Pant University of Agriculture & Technology, Pantnagar, Uttarakhand, India

✉ chakrabortymou007@gmail.com

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Abstract

Existing conventional practices for e-waste management are harmful to deal with; as a result, using an enrichment culture method, indigenous soil bacteria were investigated for e-waste treatment. In the presence of e-waste, soil bacteria were enhanced and findings were established through OD600 values. In this study, bacterial entrapment techniques (bioformulation) to increase the shelf life of e-plastic degrading bacteria isolates are provided together with their isolation and characterization. The most effective bacteria, P4, were biochemically described and immobilized using a bio-formulation made of talc and charcoal, and maintained at 25°C and 4°C for two weeks. As a result, the talc-based formulation might be beneficial for storing and managing polymer-degrading bacteria on a wide scale to reduce solid waste disposal. More research is needed to determine the bacterium's action at e-waste contaminated site.

Keywords Acrylonitrile-butadiene styrene, bioformulation, cell viability, enrichment method, e-polymer

Introduction

In this century, the global revolution in the electronics sector has coincided with a devastating change in human lives, resulting in huge outmoded electronics or e-waste. This industry is expanding at an exponential rate [1-2]. The amount of electronic garbage produced has skyrocketed. The term Waste Electrical and Electronic Equipment (WEEE) refers to obsolete, out-of-date, or abandoned electronic items. E-waste is a rapidly growing environmental problem that comprises both precious and harmful components that need unique handling and recycling techniques [3]. Plastics are neither the major residue nor the most pollutant in electronic trash, but they take up a lot of space owing to their low density and component forms. They account for around 17% of the WEEE stream [4]. On the other hand, they are generally thermoplastics that may be reprocessed and recycled.

Traditionally, there are four categories for recycling plastics, and e-waste plastics can be classified in the same way [5]. Primary and secondary technologies both deal with the mechanical recycling of polymer waste into a product that has properties close to the original product. It's possible that this mechanical recycling method [6] is the one that's employed the most to recycle plastics [7]. In the case of e-waste plastics, tertiary processes mostly consist of extracting brominated flame

retardants (BFRs) from the polymers via chemical or solvent treatment, followed by processing the plastics into useable goods. Hazardous substances like dioxins are a serious problem when employing e-waste plastics as a feedstock for waste to energy operations. The majority of quaternary recycling solutions for e-waste are still at the research stage. Some microbes, on the other hand, have shown a significant capacity to break down extraordinarily stable e-polymers with the help of intracellular and extracellular proteins stimulated by a stressful environment. When e-plastic is broken down, all isolates release extracellular depolymerase enzyme, according to Sekhar et al., [3]. Zu et al., [8] validated this discovery by observing that Ochrobactrum sp. T released more extracellular and internal enzymes as tetrabromobiphenol A was degraded.

However, environmental parameters that affect microbial development, such as temperature, pH, and nutrient availability, have a significant impact on the path of microbial degradation. Bio-augmentation (feeding pre-grown microbes to polluted soil or lab-cultured) and bio-stimulation (supplying nutrients that will boost the growth of microorganisms) are two regularly utilised ways to speed up microbe development and improve microbial degradation efficacy. Bioformulations provide microorganisms an edge in terms of viability. As a result, it has been proposed to use carriers to transport microorganisms to soil for the aim of remediation [9-11]. However, the focus of this paper is on bioremediation of e-waste polymers employing indigenous bacterial strain. Because the viability of inoculums in an appropriate formulation over a certain period of time is critical for the technology's commercialization. For this objective, the present work has been undertaken with a goal to producing bacterial isolates in carrier-based formulations for e-plastic bioremediation.

Methodology

Soil sample and e-plastic collection

For the isolation of bacterial strains, soil samples were obtained from places polluted with e-waste at a depth of 5-10 cm below the earth (probability of obtaining indigenous microbial plastic-survivors is substantially higher). Randomly disposed off old television casings containing ABS (Acrylonitrile-butadiene styrene) plastic were collected and manually dismantled to get the plastic portion, which were then grinded to make granulated e-waste. For degradation experiments granulated e-plastics were thoroughly surface sterilized and mixed with soil sample and left for a period of 6 months. Thereafter isolating bacteria from soil samples was done using enrichment methods and the serial dilution approach.

Media

Routine culture medium, such as HiMedia's Nutrient agar (NA), were utilised in the isolation procedure. The minimal media (devoid of glucose) composition used was as follows (g): NH_4NO_3 (1), KH_2PO_4 (1), Na_2HPO_4 (1.8), $MgSO_4$ (0.05), $CaCl_2$ (0.0002), $FeSO_4.7H_2O$ (0.0003), $MnSO_4.4H_2O$ (0.0002) and Na-EDTA (0.0004) dissolved in 250 ml distilled water and pH maintained at 7. The minimal media mixture was thereafter sterilized by autoclaving for 20 minutes at 121°C. To evaluate biodegradation, previously weighed sterilized pieces of plastic were put to the minimal medium as the sole source of carbon. Talcum steatite, talc fine powder, and hydrous magnesium silicate were employed in HiMedia's talc.

Enrichment and isolation of e-plastic degrading microbes

1.5 g of soil sample and 1.5 g of e-waste granules were dissolved in 250 ml autoclaved minimum medium in a conical flask. For a week of incubation, the flask was placed in a shaker at 30°C and 120 rpm. The flask with no soil inoculum was used as a negative control. After incubating for seven days, 1 mL of the enriched soil sample was mixed with 9 mL of double purified water. Up to 8 dilutions were made using this serial dilution method. Each dilution's sample was extracted and spread over 200 ml of nutritional agar on petri plates. These plates were then incubated at 30 °C for 24 hours.

On NA plates, bacterial colonies began to form. By streaking single colonies on nutrient agar plates repeatedly, single colonies were chosen and purified.

Screening of bacterial strains

Most efficient e-waste degrading bacterial culture was screened out by taking optical density at 600nm (OD600) at 24, 48 and 72 hours interval. MIC (Determination of Minimum Inhibitory Concentration) for the selected resistant isolates. Bacterial isolates that were able to grow on nutrient agar plates supplemented with 400 µl (measured out from e-waste stock solution) were finally selected for further study.

Biochemical and morphological characterization

Bacterial strain P4 was then streaked on nutrient agar and morphological characteristics such as cell shape, size,margin, elevation, Gram's, and surface staining were recorded after 24 hours of incubation. For biochemical characterization, catalase, gelatin liquefaction, casein hydrolysis, starch, and IMViC tests were performed.

Determination of optical density (OD600)

The selected strain showed optimum growth at 30±2 ºC in the presence of e-waste supplemented nutrient broth. Adding of nutrients results in enhanced microbial growth. In the present experiment sodium nitrate, urea and peptone have been added as growth substrates to check the augmentation in bacterial growth in nutrient broth in the presence of e-waste.

Preparation of bio-formulation

Bacterial cultures were introduced into 100 mL of nutrient broth (pH 7) and maintained for 48 hours at 30°C and 120 rpm. Afterwards, the active culture was centrifuged for 20 minutes at 10,000 rpm. The bacterial pellets were collected and uniformly homogenized under aseptic environments with 10 g sterilized talc and then dried at room temperature. The preparation of charcoal bio-formulation followed a similar approach. When the mixtures had fully dried, they were placed in sterile, airtight packages and sealed tightly to prevent contamination. The bio-formulations were kept at room temperature (25°C) and at 4°C in the fridge.

Microbial analysis

The spread plate method was used to determine bacterial colony forming unit (CFU) on nutrient agar plates by serial dilution method. Bacterial strains were extracted directly from talc and charcoal-based bio-formulations using the spread plate approach, which involved serial dilution on agar plates and incubation at 30 ° C for 24-48 hours.

Results and Discussion

Classification of the bacterial strain

After isolation, 52 pure cultures were obtained by continuous streaking and restreaking from enriched soil. Screening for 10 most efficient e-waste degrading bacterial culture was done by taking Optical Density (OD) at 600nm at intervals of 24, 48, 72 and 96 hours, that were then tested for growth in various e-waste concentrations. Amongst all of the bacterial isolates obtained, strain P4 grew the fastest on NA plates as shown in Figure 1a, supplemented with 400 µl of e-waste was then chosen for further experiments.

Biochemical analysis

In aerobic microbes, the enzyme catalase facilitates the conversion of hydrogen peroxide to water and oxygen [12]. Casein hydrolysis (Fig 1b) shows that the microbe generates a proteolytic

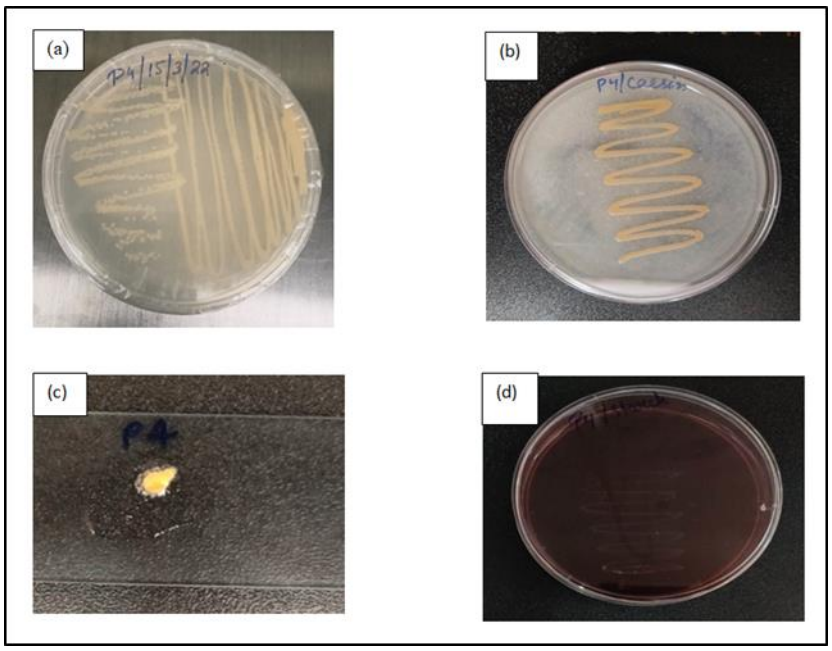

Figure 1. (a) Morphology of P4 bacterium, (b) Casein hydrolysis test result, (c) Catalase test and (d) Starch hydrolysis

exo-enzyme called proteinase (Caseinase) that can digest casein, and the gelatin test shows that the microbe can make gelatinase, an enzyme which liquefies gelatin, both of which are negative for the bacteria P4. Formation of bubbles (Figure1c) was detected as a positive outcome in the in investigation, indicating that the bacteria P4 can manufacture catalase. If the bacteria can solubilize starch, they do so in the medium, especially in areas which surrounds their growth, whereas the rest of the plate contains non-hydrolyzed starch [13]. After adding iodine solution, there is a clear zone surrounding the line of growth, indicating that perhaps the microorganism has hydrolyzed starch. Since it is unable to hydrolize starch, the bacterium P4 produces negative findings (Figure 1d) in the starch test. Morphological characteristics of bacterium P4 is depicted in Table 1. HiMedia test kits were also used to perform the IMViC test as shown in Table 2.

Table 1. Morphological characteristics of bacterium P4

SN.	Feature	Type
1	Margin	Entire
2	Cell shape	rod-shaped
3	Size	Small
4	Elevation	Convex
5	Surface	Smooth
6	Gram's stain	Positive

Variation in growth pattern of selected bacterial isolate in nutrient broth and in presence of various nitrogen sources

The OD_{600} value was obtained at definite intervals using UV-Vis spectrophotometer to monitor the bacterial growth pattern as shown in Figure 2. Addition of all the growth substrates to nutrient broth media at 30±2 °C for 48 hours resulted in the augmentation of growth pattern of the bacterial isolate as observed in Figure 3.

Table 2. IMVic test using KB001 Kit

SN.	Test name	Result
1	Indole	-
2	Methyl red	+
3	Voges Proskauer	-
4	Citrate	-
5	Glucose	-
6	Adonitol	-
7	Arabinose	-
8	Lactose	-
9	Sorbitol	-
10	Mannitol	-
11	Rhamnose	-
12	Sucrose	-

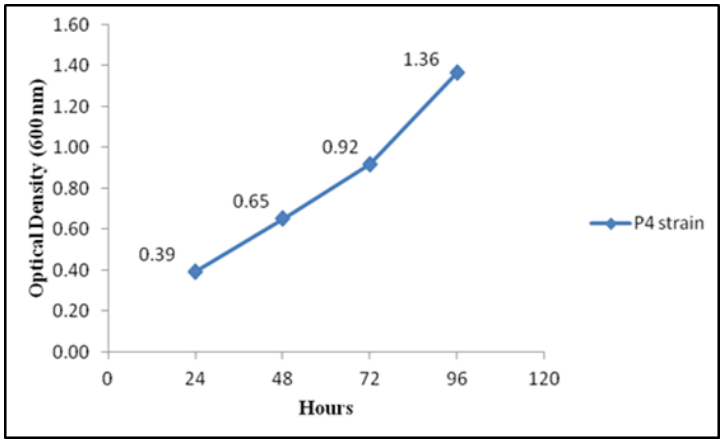

Figure 2. Growth pattern of P4 strain in presence of e-waste at definite intervals

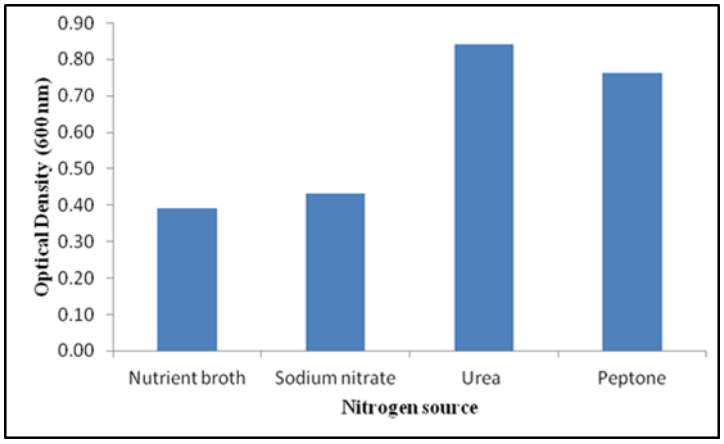

Figure 3. Enhancement in bacterial growth due to addition of growth substrate

Bioformulation study

One of the advantages of bioformulation for commercialization is its viability for a certain time period. Nonetheless, the use of a carrier-based bioformulation allows for easy transport and administration of bacteria and bacterial consortiums to specific areas [14]. Microbes were immobilized in alginate beads as part of Zommere and Nikolajeva [15] investigation into the viability of microbes. They discovered that bacteria can survive for 70 days in this entrapped state. Alginate beads were shown to be an efficient storage method by Power et al. [16]. Who also found that after 250 days of storage in alginate beads at room temperature, active *Pseudomonas fluorescens* F113 recovered 100%. The current work entraps bacteria P4 with the ability to breakdown e-polymer using 2 carriers, charcoal as well as talc. The experiment was carried out at two distinct temperatures, 4°C and 25°C, in order to determine the best substance and temperature for long-term storage. Two different temperatures 4°C and 25°C were used for the experiment. The most viable bacterial cells, according to research, were those that were trapped on charcoal and stored at 4°C. CFU count peaked on day 14th and was followed by talc bioformulation.

The bacteria have been shown to be least effective when stored in a talc-based formulation at 25°C. Therefore, it can be concluded that Talc and charcoal both showed to be excellent carriers of the e-waste degrading bacteria P4 in a 14-day trial, where, charcoal at 4 ºC was found to be the best supporting carrier. The data pertaining to the bioformulation study are presented in Table 3.

Table 3. CFU count after two weeks in different conditions

SN.	Bio formulation type	CFU mL^{-1} at subsequent time interval (days)			
		2nd day	6th day	10th day	14th day
1	Talc based bioformulation (4°C)	3.5×10^6	3.4×10^6	4.0×10^5	1.2×10^5
2	Talc based bioformulation (25°C)	2.8×10^6	3.0×10^5	8.4×10^4	8.88×10^4
3	Charcoal based bioformulation (4°C)	4.3×10^6	1.3×10^6	5.4×10^5	6.82×10^5
4	Charcoal based bioformulation (25°C)	2.5×10^6	2.0×10^6	9.8×10^4	9.5×10^4

Conclusion

Efficient and environmentally biodegradation of e-waste polymers are steadily taking on more significance because of the drawbacks of chemical and heat degradation. In this investigation, non-pathogenic e-plastic-eating strains of bacteria were successfully found and examined. The current work shows that bacteria may be used in bioformulated forms for contaminant breakdown at polluted areas. Bacteria that are transported in such linked forms rather than free form have a longer shelf life. Suitable carriers may also have the added benefit of boosting contaminant bioavailability while simultaneously offering nutrition to microorganisms. Additionally, charcoal and talc are inexpensive, readily available, and environmentally friendly materials, which address concerns about cost-effectiveness and secondary contamination. Using microorganisms with such associations to breakdown various contaminants might be beneficial. Further research is needed to assess the ability of such associations to degrade pollutants in field conditions.

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