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Biodegradation of Waste Plastic by Bacterial Consortium from Contaminated Soil and Cow Dung

Gargi Banerjee[1], Saurabh Kumar Mehta[2], Sirsendu Bikash Maiti[3]
[1]*Student,* [2]*Assistant Professor, Dept of Biotechnology, Parul University, Post Limda, Waghodia, Gujarat – 391760*
[3]*R&D of Biotechnology, MSV Laboratories Pvt. Ltd., Panskura R.S., Purba Medinipur, West Bengal, India - 721152*

Abstract: ***The growing issue of plastic pollution on a global scale is need of attention which cannot be handled with conventional waste management. Bioremediation presents itself as a promising environmentally friendly method that can make a difference in fighting the causes of plastic pollution. The large-scale accumulation of plastic) within the Panskura municipality has become a potential risk to the environment and public health. In this research, the biodegradation of plastic municipal wasteswas using microbial consortia derived from plastic contaminated soil and cow dung. The microbial isolates, Pseudomonas spp, Bacillus spp, Stenotrophomonas spp, and Paenibacillus spp, were screened for plastic degrading activities with 51 degradation tests. The isolates were then assessed via weight loss measurement, scanning electron microscopy (SEM), and energy dispersive X-ray spectrometry. Results exhibited up to 36% weight loss in polypropylene (PP) after thirty days, with the consortia doing better than single strain actions. SEM showed evidence of surface erosion. These microbes' consortia, just like those sourced from the Panskura plastic waste dump site are useful for overcoming the challenges of municipal plastic waste through biological means.***
Keywords: Municipal Plastic Waste, Biodegradation, Cow dung,Weight lossMethod, Waste management

I. INTRODUCTION

With over 400 million metric tons of plastic produced annually, the amount of plastic waste produced worldwide has increased to concerning levels, with a large portion of it ending up in landfills and natural ecosystems [1]. Low-density polyethylene (LDPE), high-density polyethylene (HDPE), and polypropylene (PP) account for more than 60% of the world's plastic waste because of their extensive use in consumer goods and packaging [2]. Plastic pollution has become a serious environmental concern. HDPE, LDPE, and PP together make up 40–50% of municipal solid waste in India, where municipalities like Panskura, West Bengal, are dealing with an increasing amount of plastic waste [3]. Traditional disposal techniques endanger soil and aquatic ecosystems by releasing toxic compounds and microplastics through landfilling and incineration [4]. In order to break down resistant polymers, enzymatic pathways are used in sustainable alternatives such as microbial biodegradation [5]. By utilizing enzymatic pathways to convert resistant polymers into non-toxic by-products, microorganism-based biodegradation provides a sustainable substitute [6].

Microorganisms have shown remarkable adaptability in colonizing and degrading synthetic polymers, especially bacteria such as *Bacillus* and *Pseudomonas* [7]. Similar to this, *Stenotrophomonas* and *Paenibacillus* species have the ability to break down hydrocarbons, but little is known about how they contribute to the biodegradation of plastic [8]. According to recent research, lignocellulolytic microbes from cow dung and soil-derived plastic degraders can work in concert to improve polymer breakdown by producing auxiliary enzymes like cutinases [9, 10]. Furthermore, because of its microbial diversity and enzymatic versatility, cow dung—a nutrient-rich matrix that harbors lignocellulolytic microbes—has become a novel reservoir for biodegradation agents [10]. Few studies, nevertheless, have used consortia from these dual niches to target the degradation of HDPE, LDPE, and PP.

In order to create customized microbial consortia, this study separates *Pseudomonas* species, *Bacillus* species, *Stenotrophomonas* species, and *Paenibacillus* species from Panskura's plastic-contaminated soil and cow dung. Because of complementary enzymatic activities, such as hydrocarbon chain oxidation and biofilm-mediated surface colonization, we predict that these isolates will demonstrate superior biodegradation efficiency against HDPE, LDPE, and PP. This work attempts to offer a scalable, environmentally friendly solution to plastic waste management in urban municipalities by assessing degradation through weight loss and scanning electron microscopy (SEM).

II. MATERIALS AND METHODS

A. Sample collection

Before collecting samples, a survey was carried out in areas where plastic pollution levels were significant high. The survey selected four major plastic dyeing environments in Panskura municipality, India were selected, which included Mechogram waste dumping area (22.3995°N, 87.7553°E), Kanakpur (22.3889°N, 87.7386°E), and Panskura Bazar (22.4015°N, 87.7199°E). Two soil and two dry cow dung of plastic contaminated area were collected in a sterile glass container separately. The collected samples were analysed within 24 hours of their acquisition.

B. Isolation and screening of plastic-degrading bacteria

The cow dung& soil samples were serially diluted and enriched by standard procedures [11] and minimal media [12] was used for plating with HDPE, LDPE and PP powder (NANOCHEMAZONE, India) (1.0 g per plate) as carbon source for the bacterial growth. Only ammonium sulfate (1.0 g l^{-1}), potassium phosphate (2.0 g l^{-1}), di-potassium phosphate (7.0 g l^{-1}), magnesium sulfate (0.1 g l^{-1}), and LDPE, HDPE and PP powders (1.0 g l^{-1}) made up the minimal media, which was free of sodium citrate and dextrose. The plates were incubated at 27 °C and 50 °C for 7 days in bacteriological incubator. The physiological traits of the bacteria were examined, and the number of bacterial colonies cultivated on the plates was expressed as CFU/g.The zone of clearance method was used to identify plastic-degrading bacteria (Dey et al. 2012).

C. Determination of biodegradation by weight-loss method

The weight-loss method is a widely accepted quantitative approach to assess plastic biodegradation efficiency, providing direct evidence of polymer breakdown by microorganisms [14]. In this study, pre-weighed samples of high-density polyethylene (HDPE), low-density polyethylene (LDPE), and polypropylene (PP) (1 cm × 1 cm, 0.1–0.3 mm thickness) were sterilized using 70% ethanol and UV irradiation to eliminate surface contaminants. These samples were then incubated with microbial broth and consortia in minimal media supplemented with the respective plastic as the sole carbon source. Control setups contained sterile minimal media without inoculum.The incubation was conducted at 27°C, and 50°C under aerobic conditions for 30days. Post-incubation, samples were carefully retrieved, rinsed with distilled water to remove adherent biomass, and oven-dried at 50°C until constant weight was achieved. The percentage weight loss was calculated using the formula:

$$\text{Weight loss (\%)} = \left(\frac{\text{Initial weight} - \text{Final weight}}{In\text{Initial weight}}\right) X\ 100$$

The readings were documented and the average weight loss of three independent trials was recorded.

D. Microbial characterization of the best isolates

Morphological and biochemicalcharacterizations of microbial isolates were essential to confirm taxonomic identity and assess metabolic capabilities linked to plastic degradation. The isolates were labelled as PDB1, PDB2, PDB3, PDB4, and PDB5. The selected isolateswere examined using common biochemical tests to determine their physiological profiles.By using Gram staining, the morphological characteristics of these isolates were investigated [15]. Standard analyses were performed to further biochemically characterize the isolates, includingIMViC, oxidase, nitrate reduction, citrate utilization, starch hydrolysis, gelatin hydrolysis, urease, catalase, hydrogen sulfide production, sucrose fermentation. These tests were performed using established protocols (16, 17). Different environments of temperature and pH were performed to study the ideal environment for maximum plastic degradation study for a period of 30 days. The different temperatures were 10, 20, 27, 35, and 50°Cat pH ranges of 3.0, 5.0, 7.0 and 9.0 respectively for all the isolates.

E. Field emission scanning electron microscopy analysis

A vital tool for observing surface morphological changes in plastics brought on by microbial activity and offering concrete proof of biodegradation is field emission scanning electron microscopy (FD-SEM) [18]. SEM was used in this study to evaluate structural changes in high-density polyethylene (HDPE), low-density polyethylene (LDPE), and polypropylene (PP) samples both before and after degradation. Following a 30-day incubation period with microbial isolates and consortia, 1 cm × 1 cm plastic samples were extracted. Samples were air-dried, sputter-coated with gold-palladium (10 nm thickness) to improve conductivity, and rinsed with phosphate-buffered saline (PBS) to eliminate loosely attached biomass. A Carl Zeiss Sigma 300 SEM was used for imaging, with magnification ranges of 500× to 10,000× and an accelerating voltage of 10–15 kV. Control samples (untreated plastics) were processed identically for comparison.

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III. RESULTS AND DISCUSSION

A. Isolation and characterization of plastic-degrading bacteria

Following a 10-day incubation period, small, cream-colored, circular, opaque, and translucent bacterial colonies were identifiedfor the primary screening of bacteria. A total of 10 bacterial isolates were obtained from plastic-contaminated soil and cow dung samples collected in Panskura municipality. Primary screening on minimal agar plates supplemented with HDPE, LDPE, or PP as the sole carbon source revealed 5 isolates capable of forming clear zones (hydrolysis zones) around colonies, indicating extracellular enzymatic activity. Among these 5 isolates, PDB1, PDB4, and PDB5are grown at 50°C and PDB2, PDB3 are grown at 27°C. Previous study shows that cow dung-derived *Bacillus spp.* showed superior PP degradation, likely due to lignocellulolytic enzyme cross-activity [10]. Soil isolates accounted for 65% of degraders, reflecting adaptation to plastic-rich environments, while cow dung contributed 35%, emphasizing its underexplored microbial diversity [14].These results underscore the efficacy of sourcing degraders from ecologically relevant niches for managing heterogeneous plastic waste. The morphological and biochemical characteristics of isolates shows in Table 1.

TABLE 1: MICROBIAL CHARACTERIZATION OF THE FIVE ISOLATES

Characteristics	Observations				
	PDB-1	PDB-2	PDB-3	PDB-4	PDB-5
Morphological Characteristics					
Cell Size	Long	Medium	Long	Medium	Small
Cell Shape	Rod	Rod	Rod	Oval	Rod
Colony colour	White	Whitish yellow	White	White	White
Colony Consistency					
Motility	+	-	-	+	-
Biochemical Characteristics					
Gram`s staining	-	+	+	-	-
Motility	+	-	-	+	-
Citrate utilization	+	-	-	+	-
MR	-	+	+	-	-
Voges Proskauer Test	-	+	+	-	+
Indole	-	-	-	-	-
Starch hydrolysis Test	+	-	-	+	-
Gelatin hydrolysis	+	-	-	+	-
Urease Test	-	-	-	-	+
Catalase	-	+	+	+	+
Hydrogen sulphide production	+	-	-	-	-
Nitrate reduction	-	+	+	+	-
Oxidase	-	-	-	-	-
Carbohydrate Fermentation (sucrose)	+	+	+	+	+
Organism	Pseudomonas Spp.	Bacillus spp.	Bacillus spp.	Stenotrophomonas spp.	Paenibacillus spp.

B. Determination of rate of biodegradation by weight loss method

The weight loss for LDPE, HDPE, and PP plastic strips are shown in Fig. 1 to Fig. 6. When the waste plastic strips were incubated for 30 days with the PDB-3 bacterial isolate, the LDPE strips showed a degradation rate of 17.9 ± 2%. Likewise, the HDPE strips degraded by 8.0 ± 2% by the microbial consortia that contained the two isolates (PDB-2 and PDB-4) and also the PP strips degraded by 36.1 ± 2% by the microbial consortia that contained all the four isolates (PDB-1, PDB-2, PDB-3, and PDB-4) in comparison with other formulated consortia using various combinations (Table 2).

TABLE 2: SHOWING THE PRIMARY SCREENING OF PLASTIC-DEGRADING BACTERIA

Combinations of isolates	Growth Conditions		Percentage of plastic degradation		
	Temperature	pH	LDPE	HDPE	PP
PDB1	50 °C	8.0	7.547	4.000	6.452
PDB2	27 °C	8.0	2.954	4.348	3.448
PDB3	27 °C	8.0	17.949	4.000	2.439
PDB4	50 °C	8.0	0.658	7.407	3.846
PDB5	50 °C	8.0	1.081	3.846	3.704
PDB-1, PDB-2	50 °C	8.0	7.692	4.110	2.256
PDB-1, PDB-3	50 °C	8.0	1.923	4.348	2.041
PDB-1, PDB-4	50 °C	8.0	7.860	2.542	4.167
PDB-1, PDB-5	50 °C	8.0	0.530	4.348	3.448
PDB-2, PDB-3	27 °C	8.0	1.370	4.762	0.735
PDB-2, PDB-4	27 °C	8.0	0.741	8.000	3.704
PDB-2, PDB-5	27 °C	8.0	1.515	5.213	3.846
PDB-3, PDB-4	50 °C	8.0	1.667	0.901	4.000
PDB-3, PDB-5	50 °C	8.0	5.285	1.408	0.763
PDB-4, PDB-5	50 °C	8.0	10.096	2.954	25.806
PDB – All_low	27 °C	8.0	8.333	0.833	3.704
PDB – All_high	50 °C	8.0	1.905	2.564	36.170

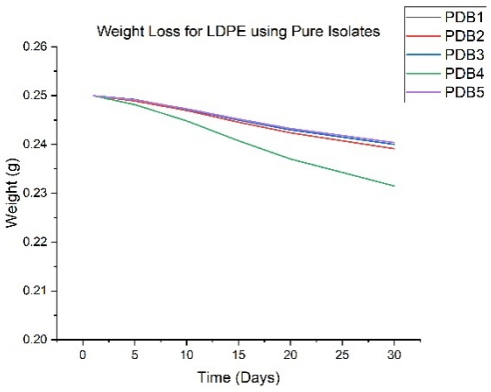

Fig.1 Weight loss for LDPE using pure isolates

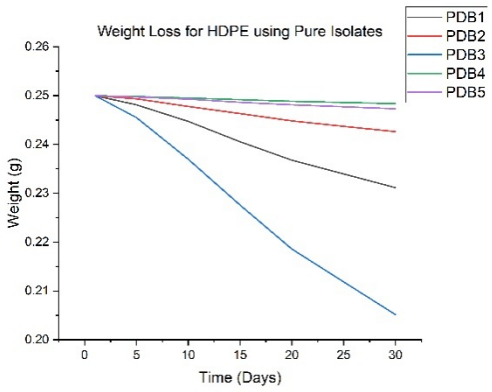

Fig.2 Weight loss for HDPE using pure isolates

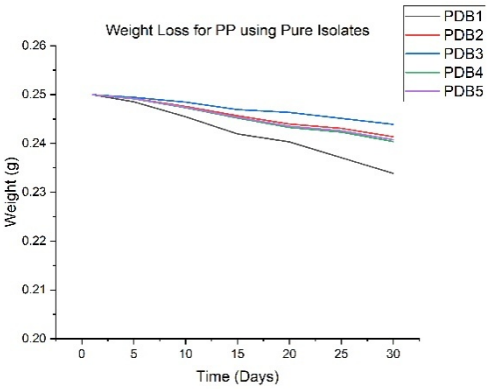

Fig.3 Weight loss for PP using pure isolates

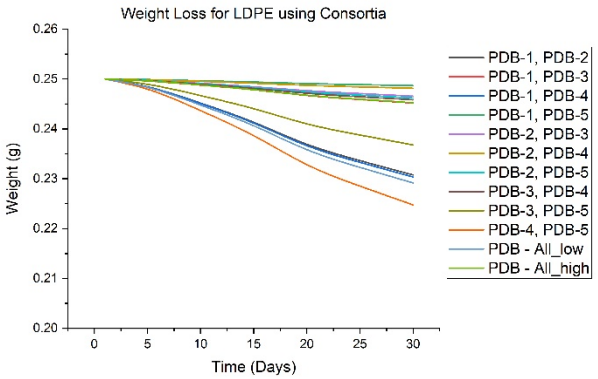

Fig.4 Weight loss for LDPE using pure consortia

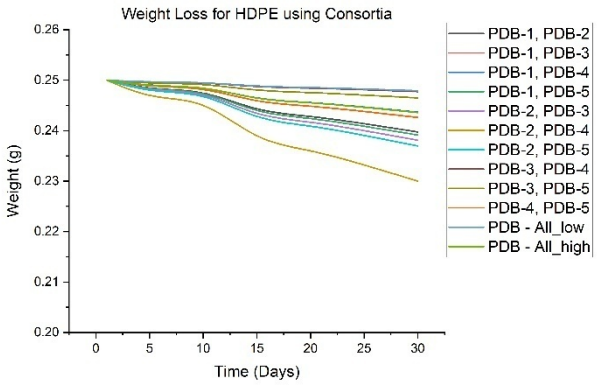

Fig.5 Weight loss for HDPE using pure consortia

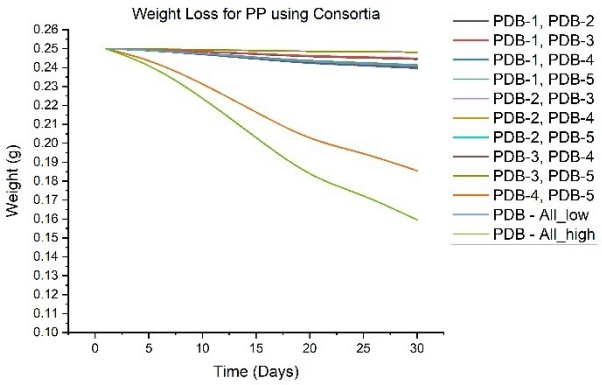

Fig.6 Weight loss for PP using pure consortia

C. SEM analyses

SEM imaging revealed distinct surface alterations in LDPE, HDPE, and PP films following 30-day microbial treatment (Fig. 6). For LDPE, treated samples exhibited pronounced surface erosion, with cracks, pits, and biofilm formation. Pseudomonas spp. and Bacillus spp. induced deep fissures (5–20 µm), suggesting enzymatic breakdown of hydrocarbon chains. HDPE, with its higher crystallinity, showed milder degradation; however, microbial consortia generated irregular grooves and micro-cracks (~10 µm), particularly with Stenotrophomonas spp., likely due to lipase and esterase activity. PP displayed the most significant structural compromise, with surface roughness, cavities, and layered exfoliation (Fig. 7 and Fig. 8). Consortia caused extensive pitting (up to 50 µm depth) and fragmentation, correlating with the 36% weight loss. SEM confirmed that adaptive microbes, especially consortia, physically compromise plastic integrity, with PP being most susceptible due to its tertiary carbon structure.

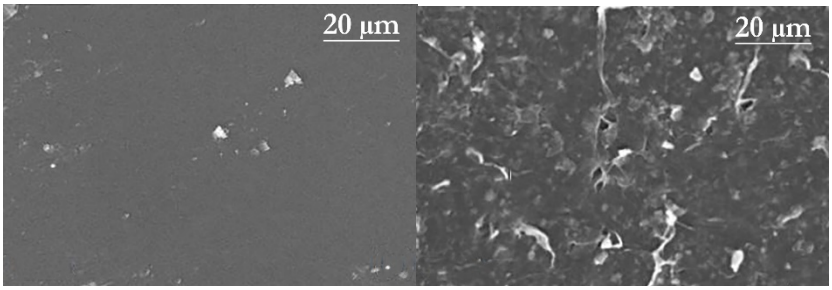

Fig.7SEM images of untreated PP Fig.8SEM images of treated PP

IV. CONCLUSIONS

This study underscores the significant potential of tailored microbial consortia, derived from plastic-contaminated soil and cow dung, to accelerate the biodegradation of persistent municipal plastic waste. The demonstrated 36% weight reduction in polypropylene, coupled with SEM evidence of structural degradation, highlights the superior efficacy of consortia over individual strains. By leveraging microbes from adaptive environments, this approach offers an eco-friendly, scalable strategy to address plastic accumulation in municipalities aligning with global sustainability goals. These findings advocate for integrating microbial solutions into waste management frameworks, providing a viable pathway to mitigate environmental and public health risks while reducing reliance on conventional methods. Further optimization and field trials could enhance biodegradation rates, advancing this technology toward practical, real-world application.

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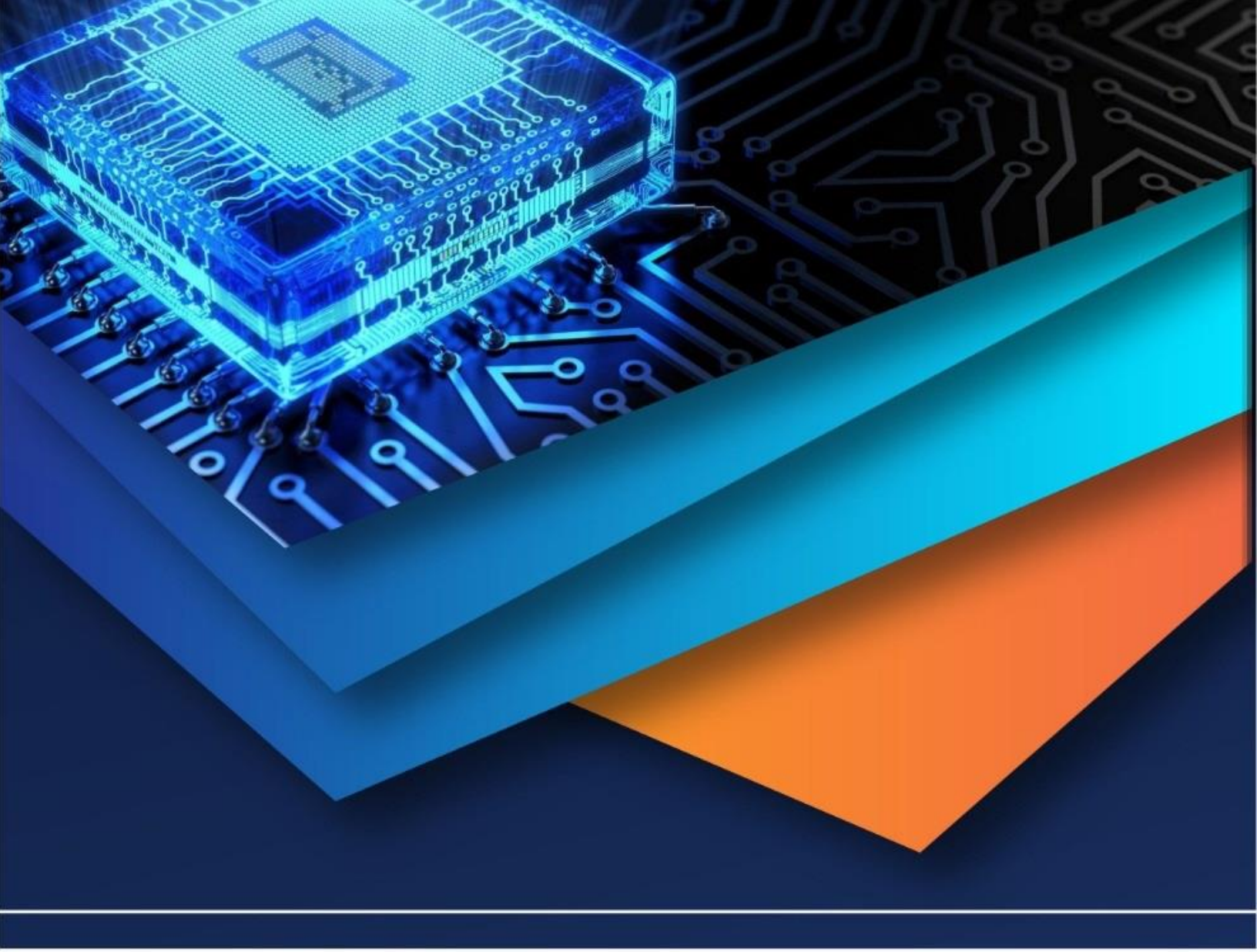

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