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Isolation of Cellulolytic Actinomycetes from Soil and its Potent Application in Decolorization of Paper and Pulp Effluent

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Abstract: ***Actinomycetes are the most profitable and biotechnologically valued prokaryotes. Actinomycetes represent a group of one of the most powerful secondary metabolite producers which possess a wide range of biological activities. In the present study, isolation of actinomycetes was carried out on Kuster's Agar plate. 16 actinomycetes were isolated from 10 different soil samples collected from different sites of College campus, Valsad. Colonies were characterized on the basis of morphological characteristics and Gram- staining. Isolates were screened for cellulase activity on Carboxymethyl Cellulose (CMC) agar plates flooded with Gram's iodine. Among 16 isolates, 12 were found positive for cellulase production. Colonies showing the highest zone of hydrolysis were selected for the decolorization of paper and pulp effluent. Strain A1, A9, A10, A11, A12, A13 and consortium of all the six selected isolates were taken for decolorization activity of Red and Black effluents respectively. The best decolorization of Red effluent was given by strain A9 (85.54%) followed by strain A1 (77.77%) whereas for Black effluent, consortium gave the best result of 56.32% decolorization followed by strain A1 (43.70%).***
Keywords: Actinomycetes, Kuster's Agar plate, Cellulase, Decolorization, Effluent

I. INTRODUCTION

Actinomycetes are gram-positive bacteria showing a filamentous growth like fungi. They are aerobic and widely distributed in nature. Actinomycetes DNA are rich in G+C content with the GC% OF 57-75% [1]. They are predominant in dry alkaline soil and belong to phylum Actinobacteria (order actinomycetales). Bergey's manual divides Actinomycetes in eight diverse groups and comprises 63 genera [2]. Actinomycetes are well recognised for their production of primary and secondary metabolites that have important applications in various fields. They are also a promising source of wide range of important enzymes, which are produced on an industrial scale. They have the ability to degrade a wide range of hydrocarbons, pesticides, and aliphatic and aromatic compounds [3]. Cellulose is the most abundant of all naturally occuring organic compounds which accumulates every year in large quantities in the form of agricultural, industrial, forest and residential wastes [4]. Cellulose is a crystalline polymer, an unusual feature among biopolymers. Cellulose chains in the crystals are stiffened by their inter and intra chain hydrogen bonds and the adjacent sheets which overlie one another are held together by weak Van-der Waals forces [5]. Bacterial cellulolysis had recently gained importance as potential source for development of commercial process because of high growth rate, wide genetic variability and adaptability and high amenability to genetic manipulation. Effective bioconversion processes of cellulosic material depend mainly on the good sources of cellulosic enzymes, the nature of cellulosics and the optimal conditions for production and catalytic activity of the enzymes [4]. Pulp and paper mill effluents have been recognized as environmental hazards for many years. The chemical composition of such effluents depends on the nature of the feedstocks, as well as the treatment procedure. The dark brown color of these effluents is mainly due to the high contents of oxidized and partially degraded lignin [6]. Reducing this color before the effluents are discharged into the natural waters is an important goal.

II. MATERIALS AND METHODS

A. Sample Collection

10 different soil samples were collected from different sites from College Campus, Valsad at the depth of 10 cm from surface. Soils were collected in polyropylene bags and its physical parameters like pH, temperature and color of samples were studied. Soil was stored under refrigerator till being used for isolation.

B. Isolation of Actinomycetes

Soil samples collected from different areas of College campus were mixed with 9ml distilled water blanks and vortexed for proper mixing. Soil was allowed to settle down and supernatant was taken for the isolation process. 1 loopfull of suspension was streaked on Sterile Kuster's Agar Plate by Four Flame method which was followed by incubation of plates at room temperature for 5-7 days. Typical Actinomycetes colonies were selected on morphological basis and gram staining as per Bergey's Manual of Determinative Bacteriology (9^{th} edn) [7] and purified on Kuster's Agar Plate by restreaking and reincubating at room temperature for 4-5 days [2]. All the isolates were then screened for cellulase activity.

C. Screening of Actinomycetes for Cellulase Activity

The cellulase producing Actinomycetes were screened by their enzymatic activity on Sterile Carboxymethyl Cellulose (CMC) Agar Plate. Isolated cultures were streaked on the sterilized CMC Agar plate containing cellulose as substrate and pH of the medium was adjusted to 7. Plates were incubated at 37^0 C for 3-4 days. After incubation, colonies were observed and confirmed by adding Gram's Iodine solution on the CMC plate and left for 4-5 minutes. Finally staining of the plates were analyzed by noticing the formation of clear zones of cellulolytic activity around the growth [8].

D. Preparation of Paper pulp

2 types of effluent were prepared

1) Red Effluent

2) Black Effluent

The effluent was prepared by cutting the paper into tiny pieces and soaking it into water for 3-4 days. Red effluent was prepared from red filter paper and Black effluent was prepared from Gujarat Mitra Newspaper. After incubation, it was blended for proper mixing. The dyes (Red and Black) present in the effluent were observed for decolorization with the help of cellulolytic isolates.

E. Decolorization of Effluent

For decolorization, 10ml effluent was taken in a tube and was tested with 0.1 ml of cellulolytic isolates with for around 1 week and decolorization activity was measured spectrophotometrically at OD 465 nm on daily basis for one week [6]. The color change was compared with the control tube. The efficiency of decolorization was expressed in terms of decolorization percentage (%) [9]:

Decolorization (%) = (initial absorbance – observed absorbance) x 100/Initial absorbance

III. RESULTS AND DISCUSSION

A. Isolation of Actinomycetes

Total 16 actinomycetes were isolated from 10 different soil samples from College campus, Valsad. The physiological characterization (pH, temperature and soil color) was carried out (Table I)

Table I
Physiological characterization of soil samples

Sr.no	Samples	pH	Temperature (^oC)	Color
1	Arts College (S1)	6	29.5	Dark brown soil
2	Nursing College (S2)	6	29	Brown soil
3	College Ground (S3	6.5	30	Light brown soil
4	Botanical Garden (S4)	6	28	Brown soil
5	B.K.M Old Office (S5)	6	28.3	Brown soil
6	Parents Water Hut (S6)	6.5	30	Brown soil
7	D.U.I.A.S M.Sc. Chem Lab (S7)	6	29	Light brown soil
8	B.Sc Micro Lab (S8)	6.5	28.5	Brown soil
9	B.K.M M.Sc Chem Lab (S9)	6	29.5	Dark brown soil
10	Waste Disposal Area (S10)	7	29	Dark brown soil

All the soil samples collected for the present study had an acidic to neutral pH ranging from 6-7.Though soil actinomycetes for the most part show their optimum growth in neutral and slightly alkaline conditions, existence of large diversity of acidophilic actinomycetes that differed morphologically and physiologically from neutrophilic species that have been reported by Khan and Willams (1975) and Williams et al., (1977). The temperature was ranging from 28-30ºC indicating organisms belonging to mesophilic group.

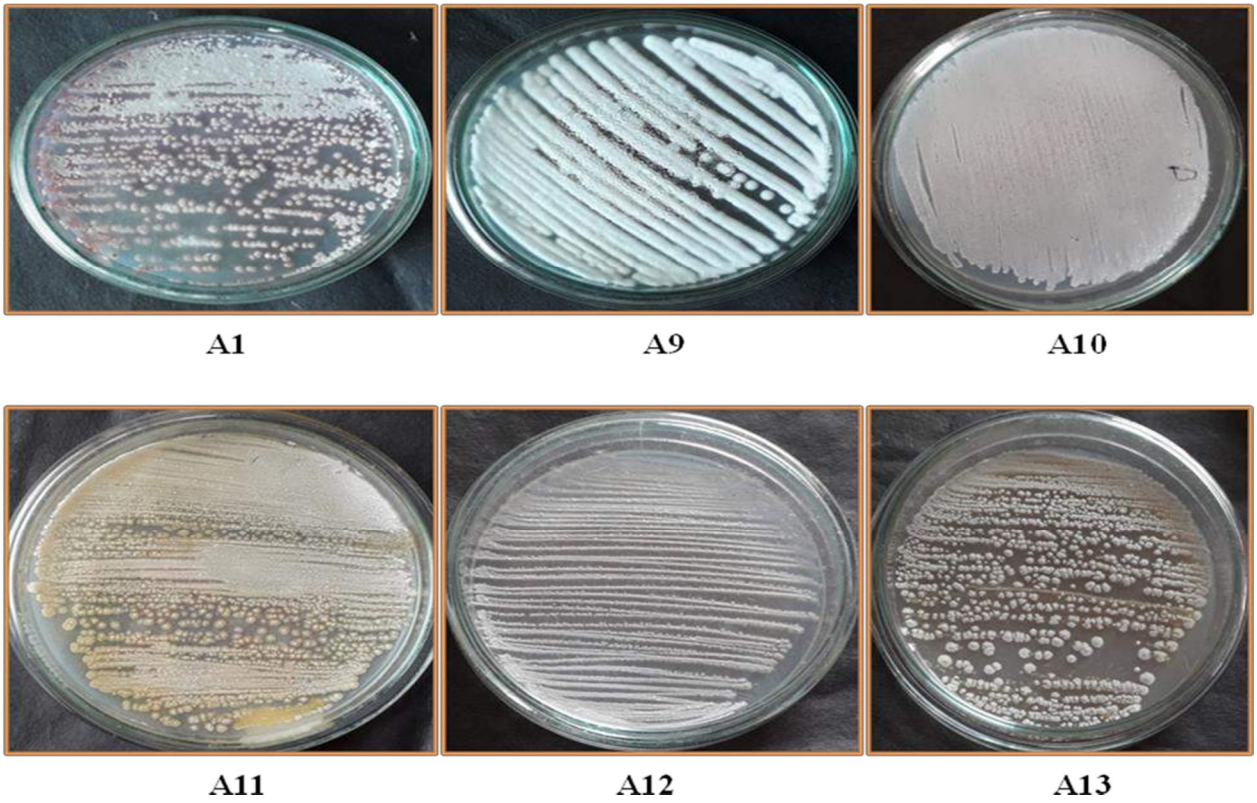

Fig 1: Actinomycetes on Kuster's Agar Plate

All the 16 isolates were Gram-positive with different filamentous growth arrangement.

B. Screening for Cellulase Activity

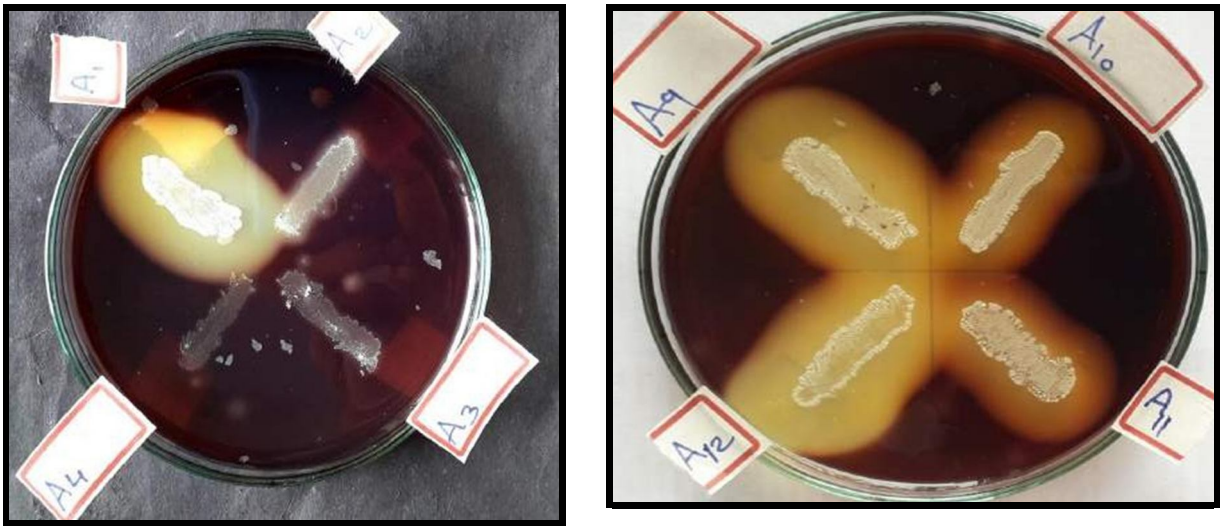

Fig 2: Cellulase Activity on CMC agar plates flooded with Gram's iodine

Table II
Results of Cellulase Activity by different isolates

Isolates	Cellulase Activity
A1	+++
A2	+
A3	-
A4	-
A5	+
A6	+
A7	+
A8	+
A9	++
A10	++
A11	+++
A12	+++
A13	+++
A14	-
A15	-
A16	+

Among 16 isolates, 12 were found positive for cellulase production. The plates were flooded with Gram's iodine and clear, distinct and prominent zone of hydrolysis was observed against the bluish-black coloration in the non-hydrolyzed part of the medium. Similar result was observed by Ramesh et al., 2008 in his work. The colonies showing the highest zone of hydrolysis were selected for decolorization activity.A1, A9, A10, A11, A12, A13 and consortium of all of them was used in the decolorization activity.

C. Decolorization of Effluent

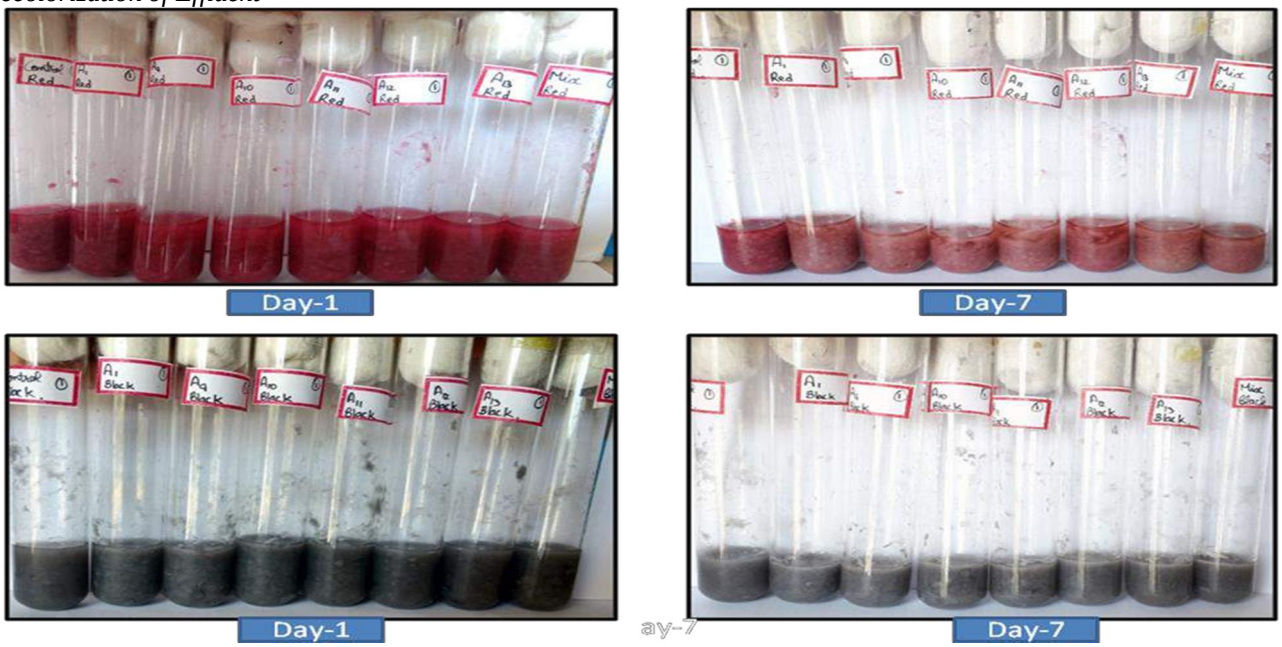

Fig 3: Decolorization of Red and Black dye in Effluent

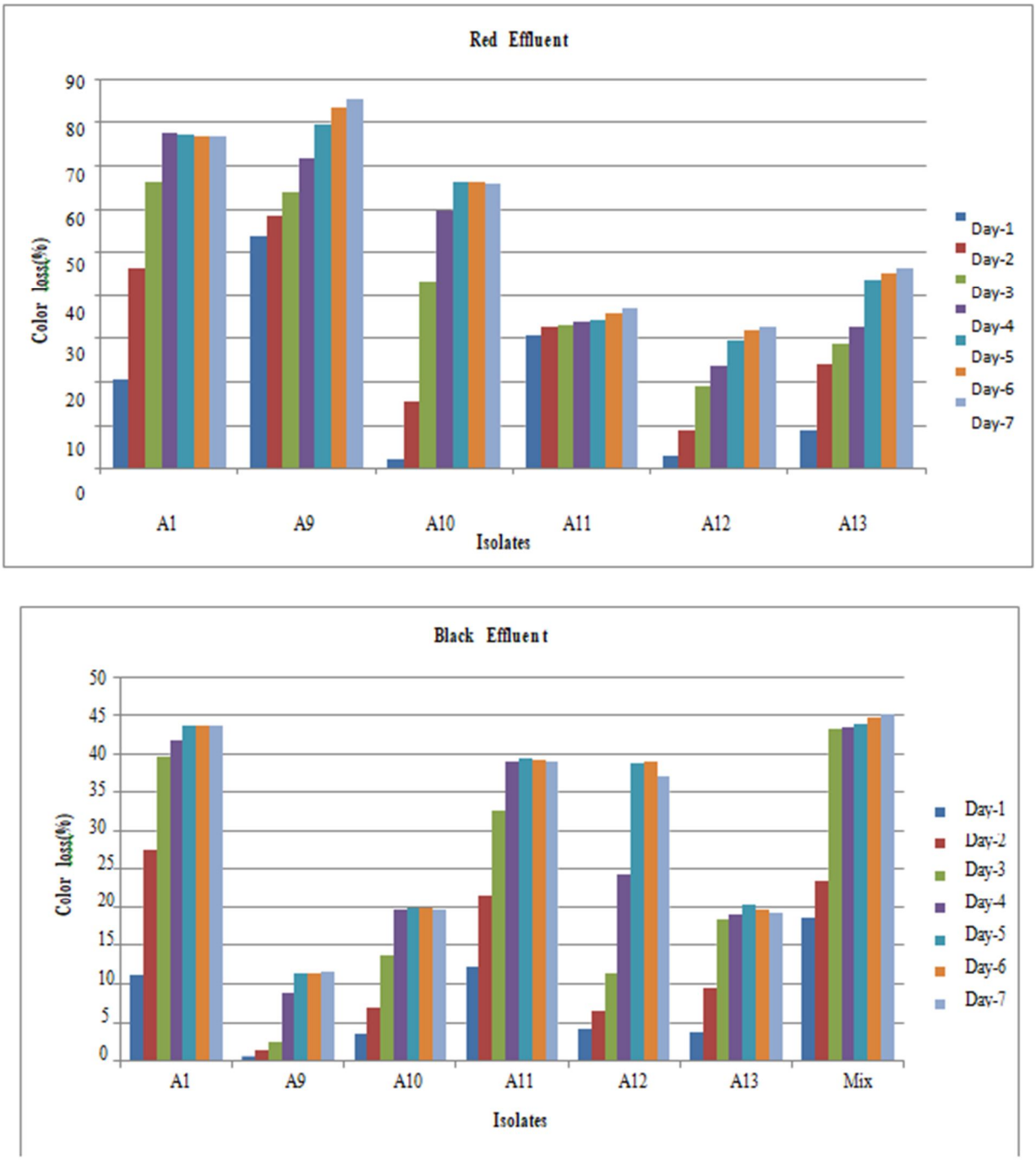

Fig 5: (%) Decolorization of Black Effluent

The color change of effluent after 7 day can be seen in figure 3. From figure 4, it can be stated that the highest decolorization of red effluent is carried out by strain A9 (85.54%) followed by strain A1 (77.77%) whereas the least decolorization is carried out by strain A12 (32.54). In case of black effluent, highest decolorization is carried out by consortium (56.32%) followed by strain A1 (43.70), whereas the least degradation is carried out by strain A9 (11.63%). Similar results were reported by [6] in which isolates were selected for their ability to decolorize the effluent in a liquid medium containing 1 (wt/vol), glycerol, 0.2%(wt/vol) ammonium sulphate, and 80% (vol/vol) effluent. He noticed that the highest levels of decolorization was achieved after the strains grew 60 to 65%.

IV. CONCLUSIONS

Actinomycetes were successfully isolated from soil samples by Kuster's Agar Medium from college campus, Valsad. 16 isolates were obtained from 10 different soil samples. Among 16 isolates, 12 were positive for cellulase activity.6 isolates giving the highest zone of hydrolysis were taken for decolorization activity. For decolorization activity, strain A9 isolate gave the highest result followed by strain A1 for red effluent. Consortium as well as strain A1 isolate gave the best result towards black effluent. Its application in decolorization of Paper and Pulp effluent has been successfully obtained using cellulase enzyme.

V. ACKNOWLEDGMENT

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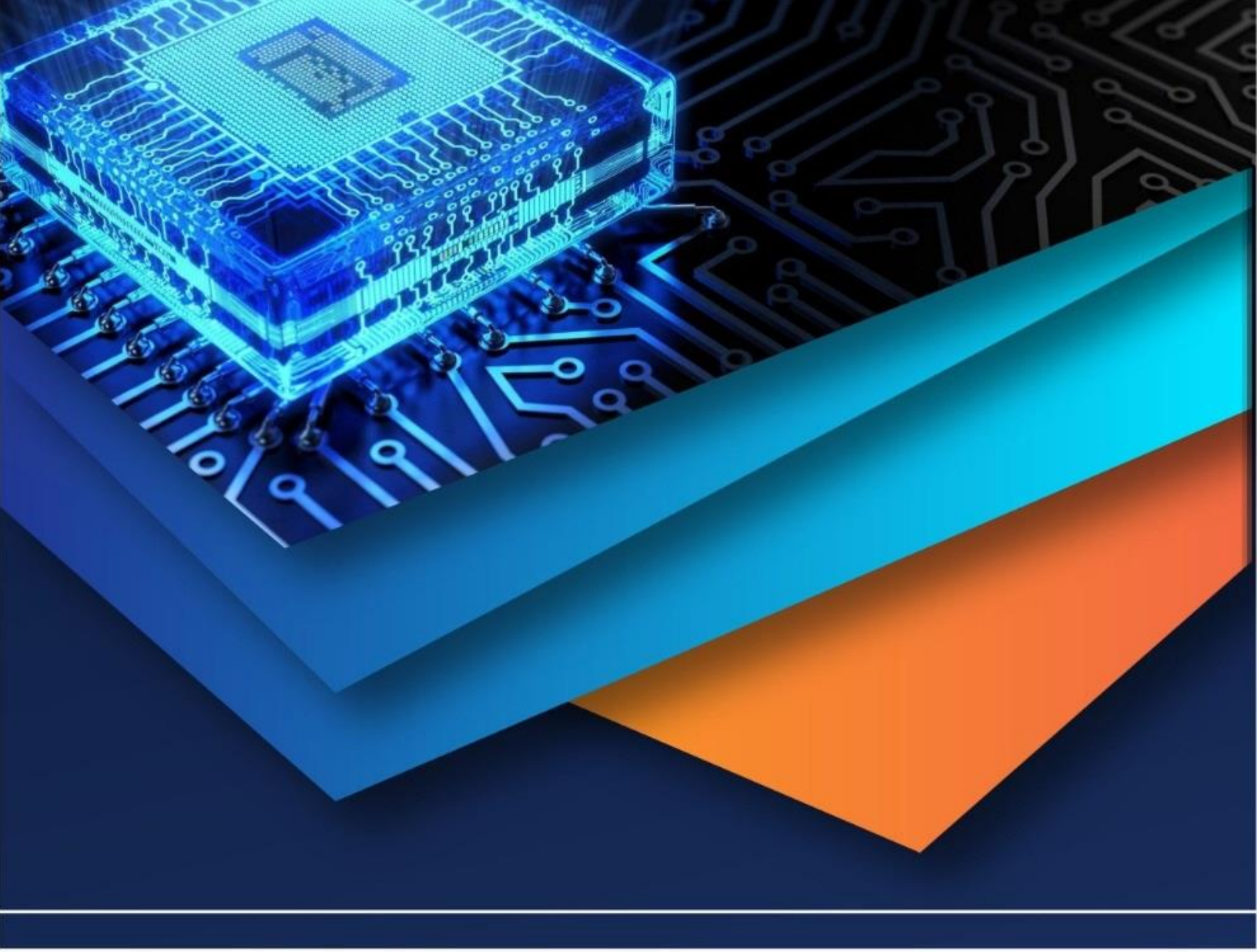

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