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Investigation on the Biosorption of Lead by Living Biomass of Aspergillus Oryzae

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Abstract: Heavy metals have been excessively released into the environment due to rapid industrialization and the presence of heavy metals in water bodies has created major environmental threat throughout the entire world. Conventional treatment methods for the removal of heavy metal ions from wastewaters are highly expensive, time consuming, less effective and are not environmental friendly because they produced large quantity of toxic chemical compounds. Biosorption is an alternative technology to conventional treatment method for the removal of heavy metal from aqueous solution. Biosorbent (biological materials) have been used as for the adsorption of metal ions from aqueous solutions. In the present study, the efficiency of living biomass of Aspergillus oryzae for the adsorption of lead metal was investigated. Living biomass of Aspergillus oryzae (100 mg and 200 mg) and contact time of 15 minutes showed that most appropriate dose and duration for the use in adsorption of lead metal ions. Results showed that, maximum adsorption capacity of 100 mg Aspergillus oryzae biomass was observed as 94.75% at 80ppm concentration. 95.218% metal removal was observed from the 80ppm metal concentration by 200 mg biomass of test fungus. The maximum specific uptake (Q value) was observed37.90 at 80ppm concentration in case of 100 mg biomass. In case of 200 mg biomass, the maximum Q value was 19.044 at 80ppm lead concentration. This study indicates that 100 mg and 200 mg living biomass of Aspergillus oryzae acts as an effective biosorbent for the adsorption of lead metal.
Keywords: Aspergillus oryzae, Biosorption, Heavy metals, Lead metal, Pollution, Q value

I. INTRODUCTION

Large scale industrialization chiefly responsible for the heavy metal pollution of soil and aqueous systems. Cu, Cr, Cd, Hg, Ni, Pb and Zn are the heavy metals present in large quantities in industrial effluents which discharged into the environment and polluting our ecosystem (Panda et al., 2014). These heavy metals are toxic and non-biodegradable and consequently biomagnified in the environment. Low concentration of metal ions is essential for various biological processes because these ions act as cofactor for various enzymes. But their threshold concentrations act as toxicant to the biological system. Metal ions are biomagnified and may entered into the population of living being through food chain, resulting serious health problems in the living being. Various morphological, cytological and physiological changes occurs in the microbial communities due the presence of these heavy metals consequently microbial communities exert a selective pressure (Vermaet al., 2001; Zafaret al., 2007).Therefore the elimination of these metal ions from soli and aquatic system is highly essential. Lead is a general enzymatic inhibitor and causes various metabolic disorders . According to Environment protection agency (EPA) the permissible limit of lead in wastewater is 0.1mg/land permissible limit for drinking water is 0.05 mg/l. Lead replaces calcium from bones, so bones become fragile due to the removal of calcium content by lead. In young children ,it can cause mental retardation and damages of brain activities hence, its removal become essential from synthetic wastewaters.

Chemical precipitation ,lime coagulation, ion exchange, reverse osmosis and solvent extraction are the commonly used procedure for the removal of metal ions (Rich and Cherry, 1987). Iram et al 2012reported that when the concentration of the heavy metal ion is low (1 mg/l to 100) then chemical precipitations methods for their removal are inefficient or very expensive. These physio-chemical techniques are costly, time consuming and their metal binding properties are not specific (Price et al. 2001).Most of these techniques are very expensive for implementation and are not eco-friendly. Khan et al. (1997) reported that the inorganic and organic waste degraded and transformed biologically by bioremediation and it can serve as the alternative methods for decontamination of heavy metals. Biological treatment methods or bioremediation techniques can be used as an alternative method for the removal of metal contaminants. Haung et al(1991) reported that through bioremediation process organic and inorganic waste biologically degraded by bacteria, fung or plants.

The removal of these metal ions is much more taken into consideration when using biomass of microbes (Volesky, 1994).Naturally fungi are having the ability to produce organic acids, extracellular proteins, various enzymes other metabolites. Fungi have the capability by which they can grow and adupt in any ecosystem and environmental condition. Fungal biomass is used as effective tool for the Biosorption or bio-detoxification of these toxic heavy metal ions(Cochrane,1958).Gadd (1993) reported that heavy metal contaminated sites are the potential source of metal resistant microorganisms. Fortunately, microorganisms have the ability to affect the reactivity and mobility of metals ions and thus can be used for the detoxification of metal ions (Tsezoset al., 1981).

Various studies showed the effectiveness of Biosorption for the detoxification of heavy metals. However, only when the cost of the biosorption process can compete with the existing technologies will it be accepted commercially. Low cost, high efficiency and regeneration of biosorbent are the major advantage of biosorption technology over the conventional methods(Kratochvil and Volesky, 1998).The cost of biomass production played a crucial role in determining the overall cost of a biosorption technique. (Kuyucak,1990).Therefore, the low-cost of biomass becomes an important factor in considering the practical implementation of Biosorption process. Sari and Tuzen(2008) used biomass of red algae (Ceramiumvirgatum) asbiosorbent for the removal of Cd(II)) from wastewater and noticed that biomass of this red alga can be used as an alternative and effective biosorbent for the detoxification of Cd ions from aqueous solution. Keskinkan et al. (2003) found that Myriophyllumspicatum can be used as an effective biosorbent for the biosorption of copper, zinc and lead.Umrania, 2006 reported that the microbial biomass can be used for the removal and detoxification of metal ions from waste waters. Anayurt et al (2009) reported that biomass of a macro fungus(Lactariusscrobiculatus) biomass can be effectively used for biosorption of Pb (II) and Cd (II) from wastewater. Apiratikul and Pavasant (2008) reported that marine algae act as potential biosorbent due to the presence of active functional groups on cell wall surfaces. Marine macro algae (as activated carbon material) having various advantages such as low cost and high efficiency of metal binding.

Carboxyl, sulphate, phosphate and amino groups are the abundant metal binding groups of cell wall of microbial biomass (Kuyucak and Volesky,1988).Biosorption by living organisms involve two steps: First, binding of metals and complex formation with the cell wall components of microbes, second, the intracellular uptake of metal ions by which metal ions are transported across the cell membrane (Costa, et.al., 1990, Gadd et.al., 1988, Huang et.al.,1990).Kapoor and Viraraghvan(1998) observed the significant role of Aspergillus niger in the biosorption of Pb, Cd and Cu ions. Species of Aspergillus has been used for the removal of metal ion from environment either by complex formation of metal ions or by the adsorption of metal ions to the component of fungal cell wall. Therefore, the present work is based on the following two objectives:

The efficiency of living biomass of Aspergillus oryzae to adsorb lead metal solutions;

The impact of lead (as lead nitrate) metal solutions of different concentrations on the biosorptive capacity of living biomass of Aspergillus oryzae.

II.MATERIALS AND METHODS

Soils samples (apparently free from pollution) were collected from agriculture fields. The upper layer of soil was removed and then soil samples were taken into the laboratory in fresh sterile polythene bags. Dilution plate method was used for the isolation of soil fungi from soil samples.

A. Preparation of fungal biomass (Biosorbent)

Aspergillus oryzaeisolated from the soil samples was selected as a test fungus for the biosorption study of lead. Pure culture of the test fungi *was* prepared on PDA (potato dextrose agar) medium.

B. Preparation of lead solution of different concentrations

Lead (as lead nitrate) was used to study the efficiency of fungal (*Aspergillus oryzae)* biomass to adsorb metal. Stock solutions of metal were prepared in a manner so as to obtain following different concentrations of metal solution i.e.,10ppm, 20ppm, 40ppm and 80ppm.

III. BIOSORPTION OF LEAD USING LIVING BIOMASS OF ASPERGILLUS ORYZAE

The method followed by Bhole et al. (2004) was adopted with slight modification to determine the efficiency of living biomass of Aspergillus oryzae for the biosorption of lead as lead nitrate.

50 ml of 10ppm solution of lead was taken into each of 6 flasks. Similarly, a set of 6 flasks were used for each of the 20ppm, 40ppm, and 80ppm concentrations of lead. Fungal biomass was then added into these flasks as under:

A. 10ppm lead nitrate solution (control i.e., without fungal biomass) (3 flasks)

B. 10ppm lead nitrate solution + 100mg fungal biomass (3 flasks)

C. 10ppm lead nitrate solution + 200mg fungal biomass (3 flasks)
D. 20ppm lead nitrate solution (control i.e., without fungal biomass) (3 flasks)
E. 20ppm lead nitrate solution + 100mg fungal biomass (3 flasks)
F. 20ppm lead nitrate solution + 200mg fungal biomass (3 flasks)
G. 40ppm lead nitrate solution (control i.e., without fungal biomass) (3 flasks)
H. 40ppm lead nitrate solution +100mg fungal biomass (3 flasks)
I. 40ppm lead nitrate solution + 200mg fungal biomass (3 flasks)
J. 80ppm lead nitrate solution (control i.e., without fungal biomass) (3 flasks)
K. 80ppm lead nitrate solution + 100mg fungal biomass (3 flasks)
L. 80ppm lead nitrate solution + 200mg fungal biomass (3 flasks)

All these flasks were then placed on a shaker for 15 minutes. After 15 minutes contact times, the content of the flasks were filtered through What man number 40 filter paper to prevent the probable interference of turbidity. The filtrate was then further processed to examine the concentration of lead remaining in the solution. Now the content of these flasks mixed together to get a composite solution for visible spectrophotometry. With the help of visible spectrophotometer, the concentration of remaining lead in the supernatant was examined at wavelength of 640-650nm.

The amount of metal bound by the biosorbent was calculated as Follows (Hussein et al. 2004)

$$Q = V (C_i - C_f) / m$$

Where,
Q = the metal (Lead) uptake (mg lead per g biosorbent),
V = the liquid sample volume (ml),
C_i = the initial concentration of lead in solution (mg/l),
C_f = the final concentration of lead in solution (mg/l),
m = the amount of the added biosorbent on the dry basis (mg),

Similarly, biosorption efficiency (R %) of the particular biomass can be calculated as:

$$R = [(C_i – C_f)/ C_i] \times 100 \%$$

The biosorptive efficiency of a particular biomass of test fungi and its particular quantity was interpreted as under:

1. 0-10	Very poor
2. 10-20	Poor
3. 20-40	Moderate
4. 40-60	Good
5. 60-80	Very good
6. 80-100	Excellent

IV. RESULTS AND DISCUSSION

The present study was conducted to analyze the biosorptive potential of living biomass of Aspergillus oryzae to adsorb Pb from the solutions of different concentrations of lead (as lead nitrate). Different amount of living biomass of fungi as biosorbent i.e., 100 mg and 200 mg were allowed the Pb from test metal solutions. After the contact period of 15 minutes, the observations were taken. The results obtained are presented in the Table 1 and Table 2 .This study showed that the living biomass of Aspergillus oryzae is effective biosorbent for the biosorption of Pb.

A glance at the table 1 and fig. 1 reveals that: After the contact period of 15 minutes 94.75% Pb could be adsorbed at 80 ppm concentration of metal solution in case of 100 mg biomass. The biosorption of Pb increased effectively from 21.80 % at 10ppm, 62.66 % at 20ppm and 88.87 % at 40ppm by the living biomass of Aspergillus oryzae.

Table 1 and Table 2 showed increase in the Biosorption percentage upto 95.218% as the biomass of test fungus increased from 100 to 200 mg. The results showed that the removal percentage of test metal(Pb) increased the increasing amount of biosorbent. The specific uptake and removal potential of metal depends on the type and amount of the adsorbent. Removal percentage of test metal increased with increasing dose of biosorbent was due to the availability of more adsorbent surfaces for the adsorption of solute.

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Table 1: Biosorption of lead (as lead nitrate) from aqueous solution of different concentrations byliving biomass (100 mg) of Aspergillus oryzae

Amount of biomass	Initial conc. of lead in the solution (ppm)	Final conc. of lead in the solution (ppm)	Amount of lead adsorbed (ppm)	Percentage of lead adsorbed	Q-value
100 mg	10	7.820	2.18	21.80%	1.09
	20	7.468	12.532	62.66%	6.266
	40	4.452	35.548	88.87%	16.274
	80	4.20	75.80	94.75%	37.90

It was found that the increasing biomass of Aspergillus oryzae(from 100 mg to 200 mg) increases biosorption percentage up to 95.218 %. In case of 100 mg biomass, the maximum specific uptake (Q value) was noticed at 80ppm concentration i.e., 37.90, followed by 16.274 at 40ppm, 6.266 at 20ppm and 1.09 at 10ppm concentrations of metal (Table 1, fig.1). In case of 200 mg biomass of Aspergillus oryzae, same pattern of increase in biosorption was also noticed as in case of 100 mg biomass of fungi. About 95.218 % metal removal was observed from the 80ppm metal concentration by 200 mg fungal biomass increased but a decrease was also noticed in the adsorption from 67.32% at 10ppm to 63.60% at 20ppm.Further increase in the conc.of test metal had a positive impact on the adsorption efficiency. At the 40 ppm conc. adsortive efficiency increased i.e.85.58% and 95.218% at 80 ppm conc.

Table 2: Biosorption of Lead (as lead nitrate) from aqueous solution of different concentrations by living biomass(200 mg) of Aspergillus oryzae

Amount of biomass	Initial conc. of lead in the solution (ppm)	Final conc. of lead in the solution (ppm)	Amount of lead adsorbed (ppm)	Percentage of lead adsorbed	Q-value
200 mg	10	3.268	6.732	67.32%	1.683
	20	7.280	12.720	63.60%	3.180
	40	5.768	34.232	85.58%	8.558
	80	3.285	76.175	95.218%	19.044

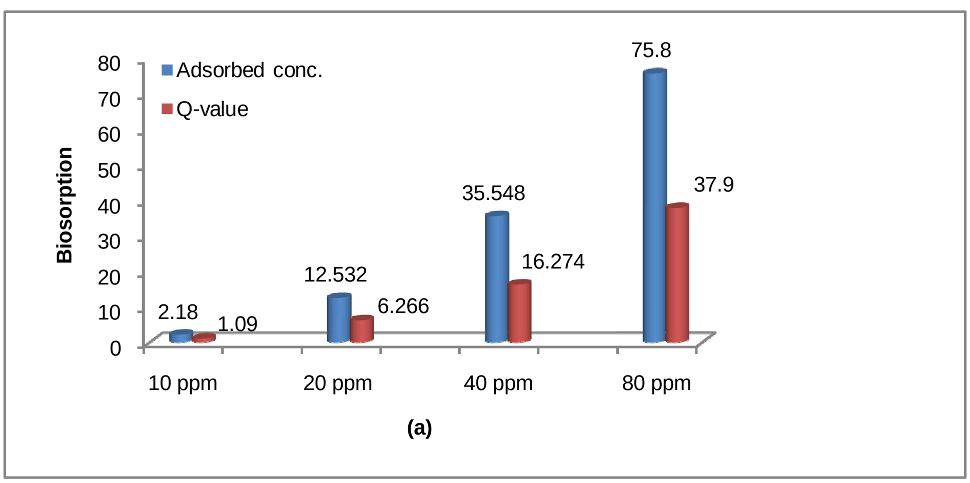

Figure 1: (a) Biosorption of lead

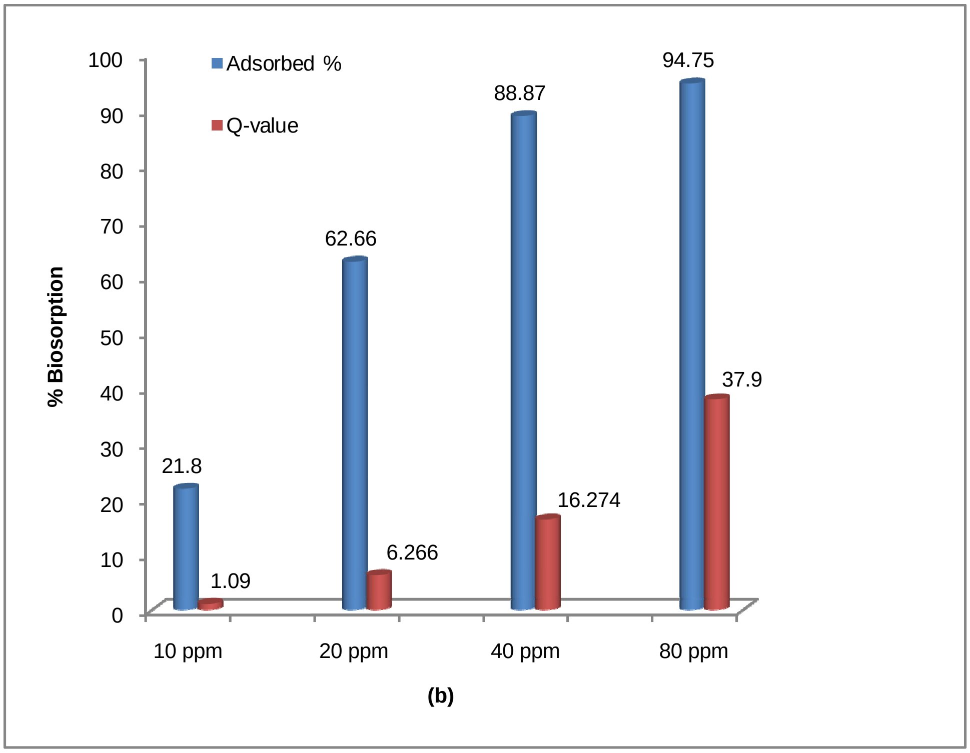

Figure 1: (b) % Biosorption profile of lead by living biomass (100 mg) of Aspergillus oryzae

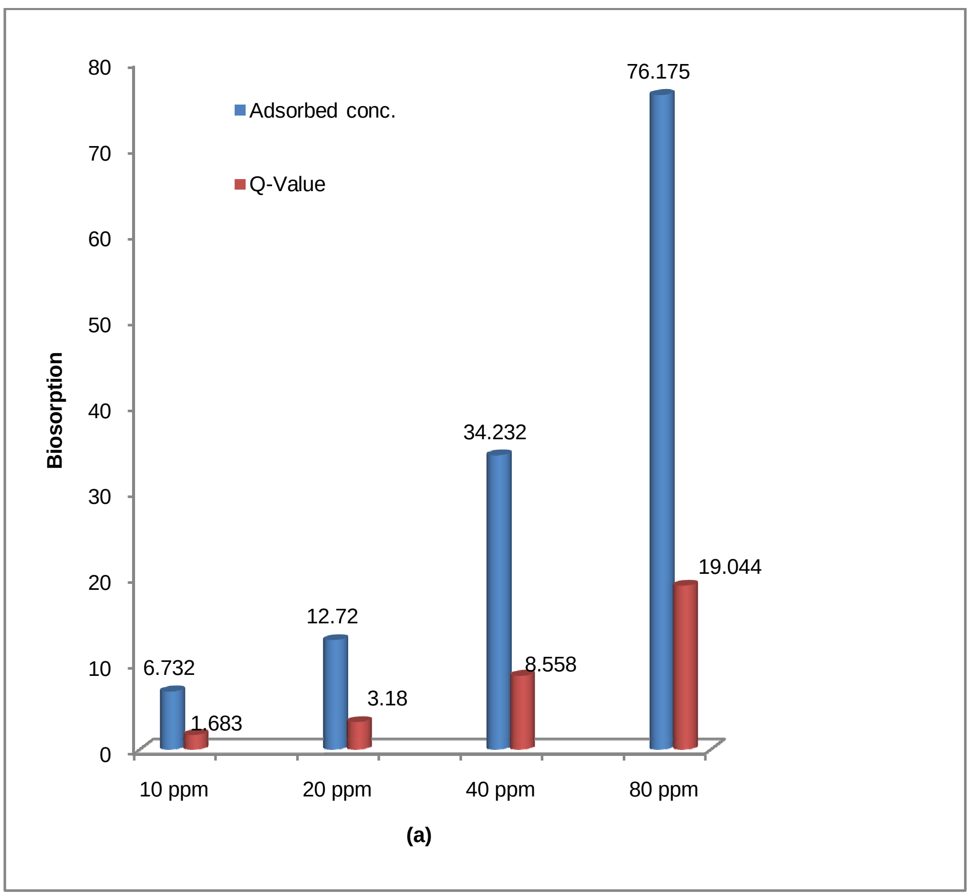

Figure 2: (a) Biosorption of lead

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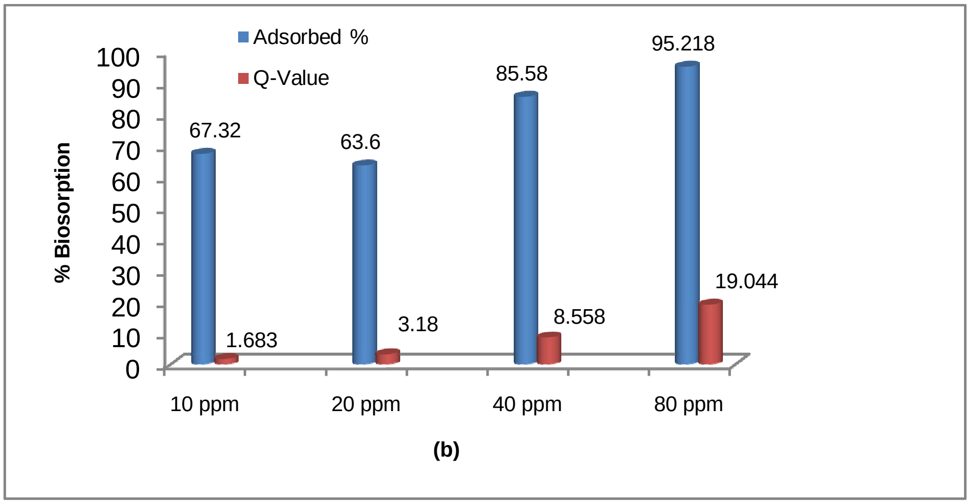

Figure 2: (b) % Biosorption profile of lead by living biomass (200 mg) of *Aspergillus oryzae*

In case of 200 mg biomass (Table 2, fig. 2) showed that the maximum uptake(Q value) 19.044 was observed at 80ppm concentration of test metal, followed by 8.588, 3.18 and 1.683 at 40ppm, 20ppm and 10ppm concentrations of metal solution respectively. The Q values indicated that increase in biomass caused a great decrease in the specific metal uptake per mg of biomass. Some worker noticed that the initial concentrations of test metal in the solution have a great effect on the sorption rate of metals by fungal biomass and it was also found that the adsorption of metal ions increase upto a greater extent with the increase in initial concentration of metal ions. These observations clearly showed that the biomass amount for the biosorption of all the concentrations of test metal at a particular incubation period can be adjusted. Chauhan et al. (2002) reported higher concentration of biomass in the solution reduces the distance between the biosorbent particles. Jaikumar and Ramamurthi (2009) reported that increasing dose of biosorbent decreases the biosorption capacity. Prabha et al (2014) reported that percentage removal of metal ion increase as the adsorbent dose increase. Abdel-Atty et al (2013) reported that the biosorption of Cd(II) and Pb(II) ions was increased with subsequent increasing the biosorbent dose of Anabaena sphaerica.

V.CONCLUSION

Heavy metals are persistent and non-biodegradable pollutants that should be essentially removed from water. Recently, biosorption have emerged as an environmental friendly, economical and effective method for the detoxification of polluted water. Present study was based on the effectiveness of living biomass of test fungus (Aspergillus oryzae) for the biosorption of lead metal ion. The present investigation indicates that the living biomass of Aspergillus oryzae is an effective biosorbent for lead metal ions and it can be used for adsorption based treatment system. Present study showed that the 200 mg (maximum dose of fungal biomass) was the most effective amount for the lead biosorption than the 100 mg fungal biomass. Present work clearly showed that biosorption efficiency increase as the amount of biomass increases and biosorption of lead metal ions by living biomass of Aspergillus oryzaeis a cost-effective and environmental friendly technology. Aspergillus oryzae biomass can be effectively usedfor managing the heavy metal pollution. Bosorption of lead metal ions by living biomass of Aspergillus oryzae is a cost-effective and environmental friendly technology. This study clearly indicates that fungal biomass is effective biosorbent for the adsorption of metal ions. The biomass materials of fungal origin contain many effective functional groups that can efficiently bind with the metal ions and positively contribute to the metal ions biosorption of heavy metals. Several studies also reported the successful application of biosorption technology for the decontamination of wastewater. Biosorption offers a impactive opportunity for the removal of metal ions from polluted aqueous system.

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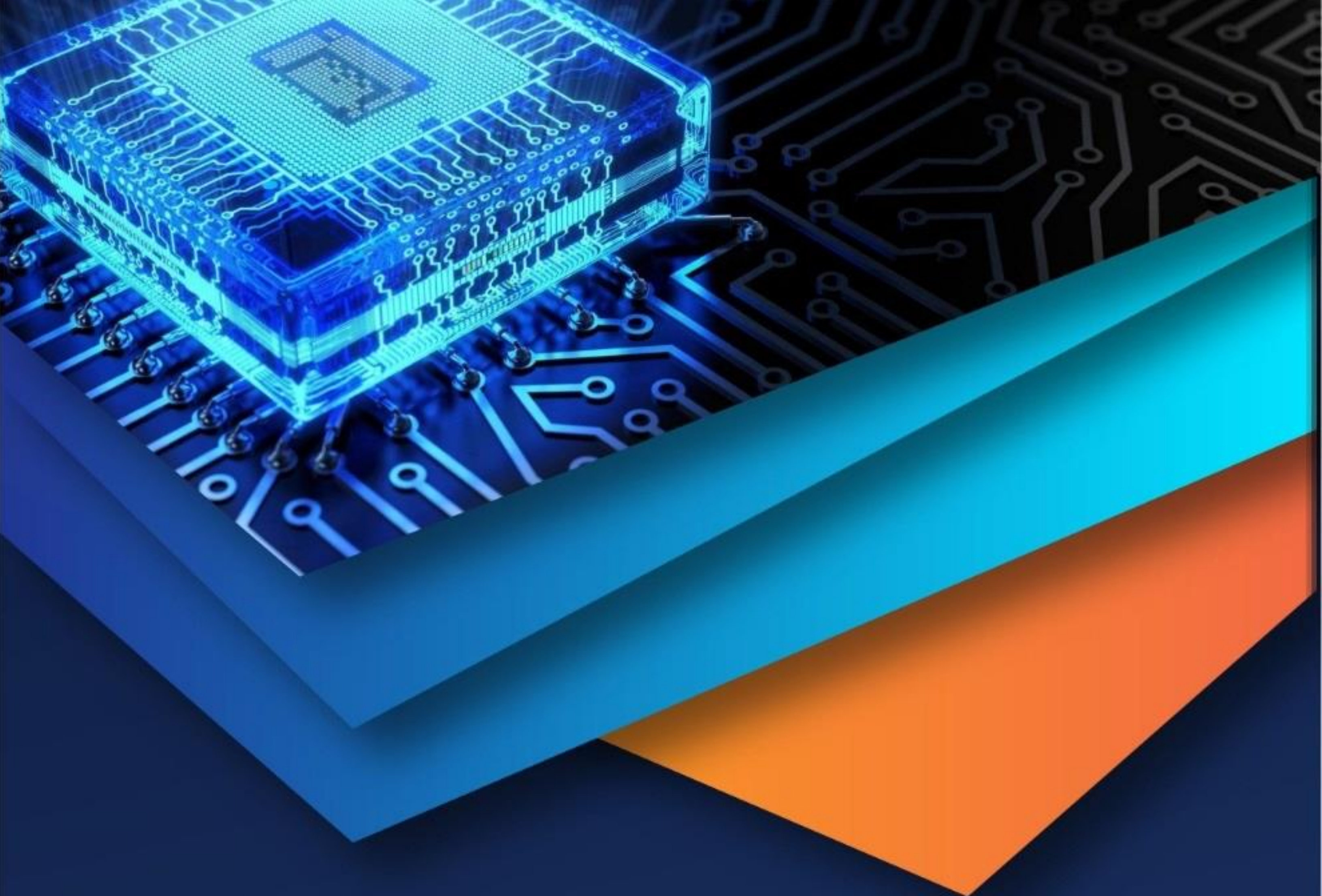

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