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Low Cost Photo Bioreactor for Marine microalga Cutivation

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Abstract*: *Microalgae growth rate changes according to the medium components, environment and characteristic features of that particular alga. Hence algal biomass production can be accelerated by giving needed nutrient medium, pH, salinity and light intensity and these can be controlled in a closed bioreactor. In this study, the lab-scale Photo bioreactor was designed to produce low-cost algal biomass. The LED lights were used to give the needed light to the algal growth. This study also evaluates the effects of different culture media in culturing Tetraselmis sp. The growth rate of algal culture was checked using haemocytometer by counting algal cells. Media like BBM, ASN III, Walne's medium and NPK seawater solution was used to study the growth curve of the culture. The produced biomass was dried using hot air and then determined the dry weight of biomass. Growth study resulted Walne's medium and NPK seawater solution was found to be best to grow Tetraselmis sp. The designed Photo bioreactor was good to culture algae under LED light and it resulted in the good biomass.

Keywords— Algal Growth curve; ASN III, BBM; Haemocytometer; LED light; NPK seawater solution; Tetraselmis sp.; Walne's medium.

I. INTRODUCTION

Algae are multi use feedstock from current scenario, which are exponentially used in various industries such as pharmaceutical, pharmacology, food, feed, cosmetics and bio energy production [1]. Numerous high nutritive marine algae like Nannochloropsis, Tetraselmis and Isochrysis have been widely employed in many aquaculture hatcheries [2-4] yielded rotifers with mixed algal culture also gives significant improvement in their quantity and qualitatively [5-10]. Algal species has the capability of producing different amount of lipids can be converted into bio energy in different media and different environmental conditions [11-13]. Since it is used in various industries; the biomass production of them is considered to be the major part, in order to utilization [14]. In biomass production, increasing the biomass is affected by various factors like culture's environmental condition and properties were affected by nature of nutrient medium and also algae shows different growth rate in different medium [15-18]. Around the globe, algal biomass was produced using various media with help of different culture methods. From those, two methods are used by the majority which are open pond system and closed Photo bioreactor. The Photo bioreactor is a basic system, where all the necessary things will be provided to enhance the algal growth since it is a closed system, which will control the environmental contaminant and also enhance the growth [19-20]. There are many industries uses the Photo bioreactor to produce algal biomass, which makes the algal biomass production costlier and most of the lab-scale Photo bioreactor also being costlier, it was decided to make a lab scale Photo bioreactor using the locally available materials. The aim of this work was to build the bioreactor at low cost and to determine the suitable growth medium.

II. MATERIALS AND METHODS

A. Sample collection and Culture Development

Algal samples were collected from Swamithope saltpan (Kanyakumari District, Tamil Nadu. India). The Culturing methods were followed described by [21]. From the collected algal samples, Tetraselmis sp. was isolated using streak plate method [22]. The algal strain was maintained in agar Walne's medium with pH 8 and Salinity 3.5% (35ppt). For producing biomass the alga was initially cultured in 50 mL conical flask using liquid Walne's medium and further scaled up to 2000 mL culture which was periodically shaken for proper nutrient mixing. Then the culture was inoculated in 20 litre Photo bioreactor (10 litre working volume).

B. Growth study and media optimization

Alga was cultured in 1000 mL conical flasks (500 mL working volume), which were added with different media i.e. Walne's medium, NPK seawater solution, BBM and ASN III. These media were further inoculated with the algal mother culture with a concentration of 0.10 with a optical density of 620 nm and placed in a photoperiodic culture racks. The culture racks were provided with 2000±05 lux unit (Using LED tube lights), light intensity and the temperature of the culture room was maintained at 24±1°C.

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Two days ones the cultures were taken and the cells were counted using Haemocytometer. Based on that, the growth curve was plotted against the time taken by the culture to grow [17]. In the 21st day of culture study, the biomass was harvested and then the wet weight and dry biomass was calculated. Considering this study resulted that the best two media were selected for the Photo bioreactor study.

C. Designing the lab-scale Photo bioreactor

A simple lab-scale Photo bioreactor was designed using the locally available 20 L packaged drinking water's cane. The water cane was the basic container for the Photo bioreactor. The new cane was purchased and later it was washed thoroughly using distilled water and then dried in direct sunlight in the dust-free location. The cleaned container was then treated with UV for 30 minutes. The container's removable cap was also treated in the same way after making two holes on the top. One hole is to insert the tube for aeration/ for providing CO_2, while another one for gas exchange and also for the regular check-up (OD, Temperature and pH), which was covered with the small cotton plug. The light intensity of this study was 5000±10 lux unit and the light received by the alga was 4000±10 lux unit maintained throughout the cultivation period. Inside the culture room, a small cabin was prepared and the reactor was set up. Photoperiod was maintained 10 hours light and 14 hours dark and the room temperature was maintained 24±1°C. The culture provides with 10 hours aeration through one hole of the cap while another hole was plugged with a cotton plug.

D. Media preparation

In this experimental study totally four media were used, which were Walne's medium, NPK seawater solution, BBM, ASN III, and their chemical components were given in Table -1, Table - 2, Table - 3, and Table - 4 respectively. Even though the alga was cultured in Walne's medium (1.5% Agar Agar - Himedia) for isolation purpose, different media were tried to check their impact on the algal growth. NPK was purchased from the local fertilizer shop grounded to powder before mixing them to seawater.

E. Harvesting and Biomass Determination

Stationary and declined phase of algal biomass was harvested by adding saturated alum solution which was again centrifuged for 15 minutes at 5000 rpm. The harvested biomass was placed in a pre-weighed Petri dish (A) and then biomass was weighed with Petri dish (B). Wet biomass weight was calculated by detecting the weight of the empty petri dish from the total weight (Wet Biomass = B-A). Then the wet biomass was placed in a clean hot air oven for 5-6 hours at 40°C. After drying, the dry biomass weight was measured in the same way (Dry Biomass = C-A), whereas C is the weight of the petri dish with dry biomass.

III.RESULT AND DISCUSSION

The isolated marine alga Tetraselmis sp., was growing in abundant at Swamithope saltpan's water holding area as reported earlier [21]. The alga was tried with different culture media like ASN III, BBM and Walne's medium during isolation and purification and it was showing faster and healthier growth in Walne's medium. After several subcultures, the alga was isolated into pure culture. The culture was further grown in liquid medium and every 20 days it was further sub-cultured and scaled up. For biomass production, the media was selected after culturing alga in 500 mL media and check the results. In that study, NPK seawater solution and Walne's medium were showing the good growth rate Table - 5 and given good amount of biomass, whereas BBM was showing slower growth and less biomass. ASN III medium was resulted comparatively higher biomass than the BBM and lower than the Walne's medium and NPK seawater solution Table - 6. During the scaling up process, above 500 mL cultures were also maintained with the NPK seawater solution, which was showing as good biomass production as alga grown in Walne's medium. The 20th day culture's cell count was compared and found no major decrease in both NPK seawater solution and Walne's medium hence the NPK seawater solution and Walne's medium were used in Photo bioreactor as the nutrient medium. The Photo bioreactor was given a good result with more biomass than the alga cultured in the conical flask with periodic shaking Fig - 1.The Photo bioreactor with Walne's medium was given lesser amount of biomass, whereas the NPK seawater solution was produced higher biomass than Walne's medium Table - 7. The growth curve study shows clear 'S' shaped curve, while first 5 days of the cultures were shows slow growth and till 15th days the growth was exponential phase and then it reaches to declining phase. Figure – 2 shows the cultures in both, Walne's medium and NPK seawater solutions were in exponential phase till 17th day and then they reached the declining phase. Hence the growth rate is higher in both Walne's medium and NPK seawater solution, Figure - 3 shows wet biomass and Figure – 4 shows dry biomass was also higher than the other media. Figure – 4 stated that the high amount of 0.1251 gram of dry biomass harvested from NPK seawater solution and the lesser dry biomass 0.0910g from the BBM marine medium. The cultures in Photo bioreactor were showing much faster growth and dense culture Figure - 1 at 15th day itself hence, the culture was harvested. Figure - 5 shows the wet and dry biomass ratio from the Photo bioreactor study. In the Photo bioreactor study the both media didn't had major difference in producing biomass which was produced much higher amount of algal biomass.

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IV. CONCLUSION

This study reveals that the alga Tetraselmis sp. is growing well on Walne's medium with the pH 8 - 8.5 and the salinity 3.5% (35 ppt). The alga was good to grow well on 2000 -4000 lux unit, and at the temperature of 24°C. Alga Tetraselmis sp. was well on NPK Seawater solution. And other media used in this study also was good enough to help the growth of algae since they were prepared from natural seawater. Natural seawater can be considered the best liquid to culture the marine microalgae, by adding some major needed nutrients and vitamins. Hence this study concluded that the developed Photo bioreactor is good in producing low-cost algal biomass for lab-scale studies.

V. ACKNOWLEDGEMENT

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Table -1. Walne's medium (prepared in sterilized seawater)

	Stocks solutions	Compounds per 100 ml
1.	Trace metal solution (TMS)	
	$ZnCl_2$	2.1 g
	$CoCl_2.6\ H_2O$	2.0 g
	$(NH_4)_6Mo_7O24.4\ H_2O$	0.9 g
	$CuSO_4.5\ H_2O$	2.0 g
	Conc. HCl	10 mL
2.	Vitamin solution	
	Vitamin B_{12}. (Cyanocobalamin)	10.0 mg
	Vitamin B_1 (Thiamine.HCl)	10.0 mg
	Vitamin H (Biotin)	200.0 µg
3.	Nutrient solution	
	$FeCl_3.6\ H_2O$	1.3 g /l
	$MnCl_2.4\ H_2O$	0.36 g /l
	H_3BO_3	33.6 g /l
	EDTA(Disodium salt)	45.0 g /l
	$NaH_2PO_4.2\ H_2O$	20.0 g /l
	$NaNO_3$	100.0 g /l
	1. Trace Metal Solution	1.0 mL/l
	Final Medium	For 1000 mL
	2. Vitamin solution	0.1 mL
	3. Nutrient solution	1 mL
	Sterilised Seawater	Make up to 1000 mL

Table -2. NPK Seawater solution (prepared in sterilized seawater)

	Compounds	Quantity g/1000mL
1.	Urea	2
2.	Superphosphate	1
3.	Potash	1
4.	Vitamin solution (as Walne's medium)	0.1mL

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Table -3. BBM Medium (prepared in sterilized seawater)

	Stock	*Stock solution*	*mL/L*
1.	KH_2PO_4	7 g/400 mL	10 mL
2.	$CaCl_2 \cdot 2H_2O$	1 g/400 mL	10 mL
3.	$MgSO_4 \cdot 7H_2O$	3 g/400 mL	10 mL
4.	$NaNO_3$	1 g/400 mL	10 mL
5.	K_2HPO_4	3 g/400 mL	10 mL
6.	NaCl	1 g/400 mL	10 mL
7.	$EDTA \cdot 2H_2O$	50 g/L	1 mL
	KOH	3 g/L	
8.	$FeSO_4 \cdot 7H_2O$	4.98 g/L	1 mL
	H_2SO_4 (concentrated)	1 mL/L	
9.	Trace Metal Solution		1 mL
	$MnCl_2 \cdot 4H_2O$	1.44 g/L	
	$ZnO_4 \cdot 7H_2O$	8.82 g/L	
	$Na_2MoO_4 \cdot 2H_2O$	0.7 g/L	
	$CuSO_4 \cdot 5H_2O$	1.57 g/L	
	$Co(NO_3)_2 \cdot 6H_2O$	0.49 g/L	
	$MnCl_2 \cdot 4H_2O$	1.44 g/L	
10.	H_3BO_3	11.42 g/L	0.7 mL
11.	Vitamin Solution (optional)		1 mL
	Vitamin B_{12} (Cyanocobalamin)	0.0001 g/L	
	Biotin	0.0001 g/L	
	Thiamine	0.0200 g/L	

Table – 4. Chemical compounds of ASN III medium (prepared in sterilized seawater)

	Chemical compounds	g/1000mL
1.	$MgSO_4$ x $7H_2O$	3.5 g
2.	$MgCl_2$ x 6 H_2O	2.0 g
3.	CaC_{l2} x 2 H_2O	0.5 g
4.	KCl	0.5 g
5.	Citric acid	3.0 mg
6.	Fe-Amm-Citrate	3.0 mg
7.	EDTA	0.5 mg
8.	A-5 Trace Metals	1.0 ml
	H_3BO_3	2.86 g
	$MnCl_2$. 4 H_2O	1.81g
	$ZnSO_4$. 7 H_2O	0.222 g
	Na_2MoO_4 . 2 H_2O	0.039 g
	$CuSO_4$. 5 H_2O	0.079 g
	$Co(NO_3)_2$. 6 H_2O	0.049 g
9.	$NaNO_3$	0.75 g
10.	K_2HPO_4 . 3 H_2O	0.75 g
11.	Na_2CO_3	0.02 g
12.	Vitamin B_{12}	10.0 mcg

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Table -5. Growth rate of culture in various media

	day 1 $*10^4$ cell/mL	day 3 $*10^4$ cell/mL	day 5 $*10^4$ cell/mL	day 7 $*10^4$ cell/mL	day 9 $*10^4$ cell/mL	day 11 $*10^4$ cell/mL	day 13 $*10^4$ cell/mL	day 15 $*10^4$ cell/mL	day 17 $*10^4$ cell/mL	day 19 $*10^4$ cell/mL	day 21 $*10^4$ cell/mL
Walne's medium	5.5	9.55	21.625	34.25	83.625	145	201.25	282.625	314.75	333.25	335.125
NPK Seawater	5.25	9.75	21.25	34.625	83.05	139.75	200.125	279.375	336.25	365.5	393.5
BBM	4.52	9	20.75	33.65	82.35	137.45	194.5	236.25	268.35	270.45	267.95
ASN III	4.76.	9.35	21	33.7	82.95	137.5	197	240.75	272.05	275.25	271.95

Table -6. Wet and dry biomass from various media in growth study

S.No.	Growth medium (pH-8; 3.5% saline)	Biomass wet weight (g/ 500 mL)	Biomass dry weight (g/ 500 mL)
1.	Walne's medium	2.1637	0.1237
2.	NPK seawater solution	2.1712	0.1251
3.	BBM	1.5038	0.0910
4.	ASN III	1.7805	0.1089

Table -7. Dry and wet biomass of culture in Photobioreactor study

S.No.	Growth medium (pH-8; 3.5% saline)	Biomass wet weight (g/ 10000 mL)	Biomass dry weight (g/ 10000 mL)
5.	Walne's medium	183.38	12.1237
6.	NPK seawater solution	189.5	14.2589

Fig. 1 Biomass production using Photobioreactor

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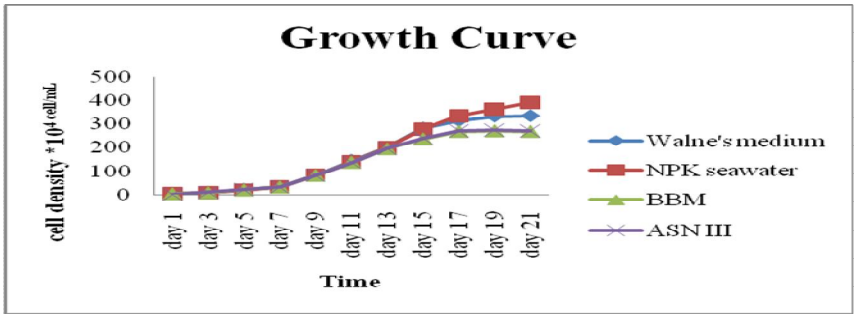

Fig. 2 Linear Graph showing the growth curve of alga grown in different media

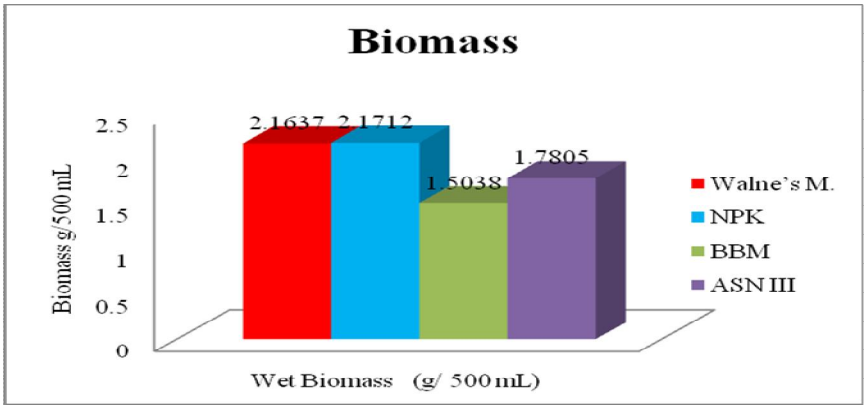

Fig. 3 Comparison of wet biomass harvested using different media

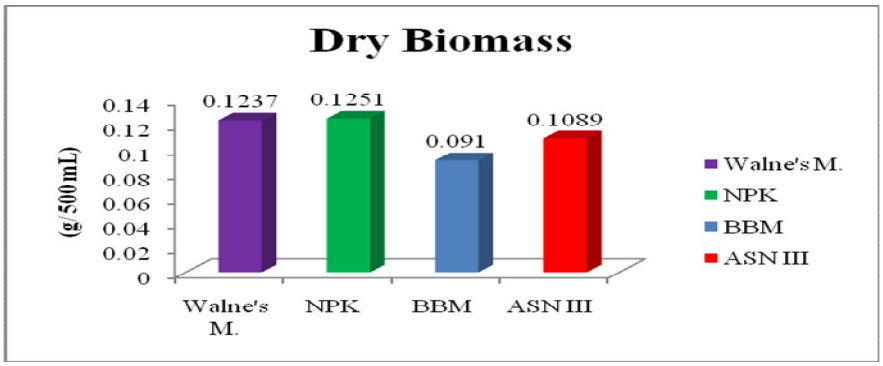

Fig. 4 Comparison of dry biomass harvested using different media

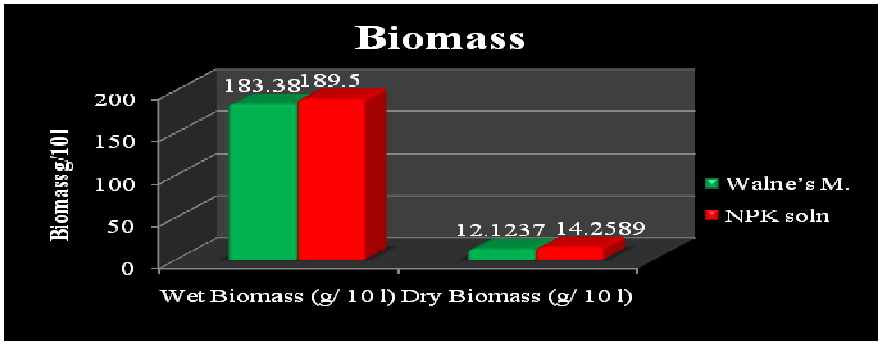

Fig. 5 Graph showing the comparison of wet and dry biomass harvested from Photobioreactor

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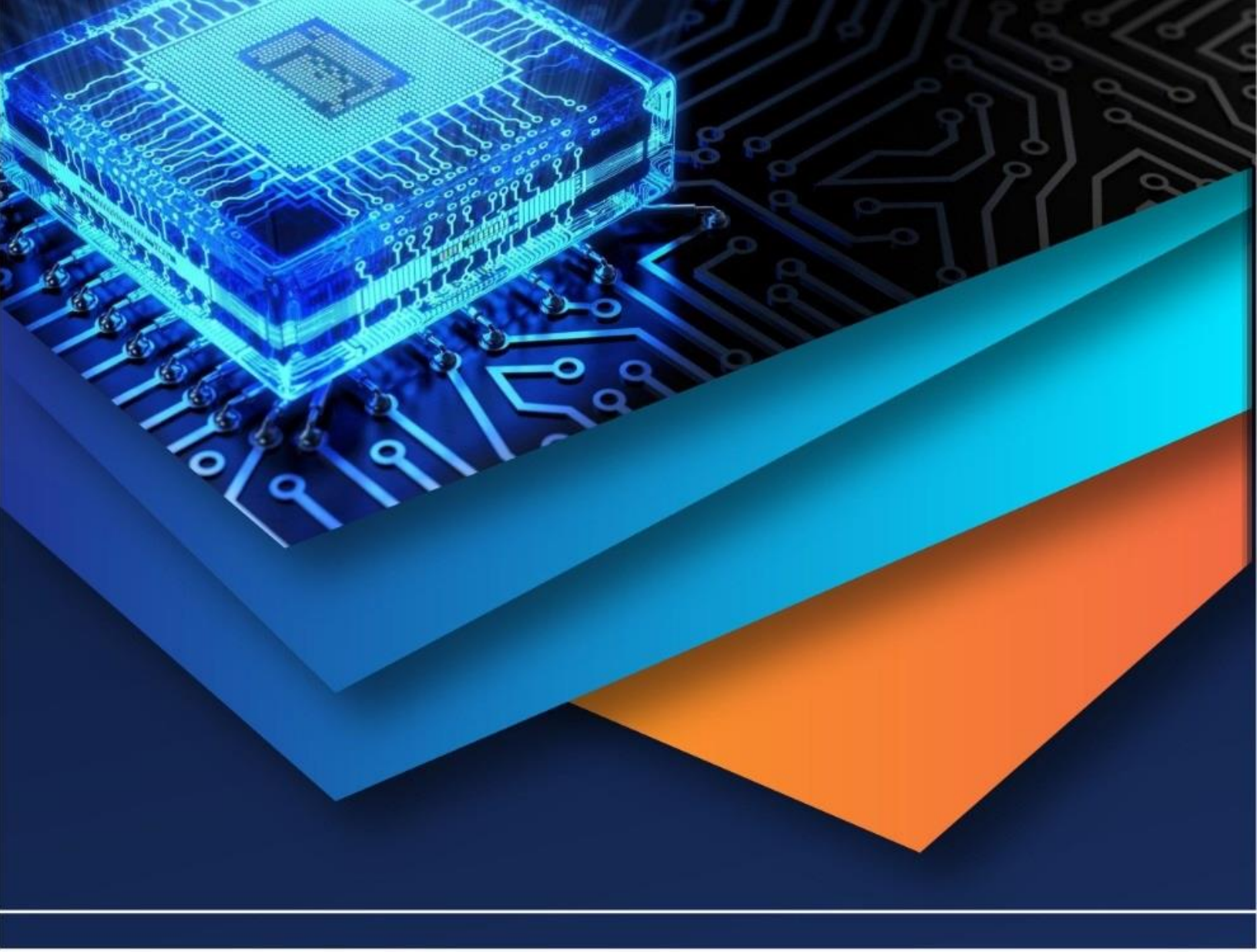

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