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Bioethanol Produced from Dovyalis Caffra (Kei-apple) by Fermentation with Yeast (Saccharomyces Cerevisiae) – Characterization, Mass & Energy Balance

M. A. Azaz Ahmed[1], Ambedkar Yedira[2] Wondalem Misganaw[3]
[1, 2, 3]*Department of Chemical Engineering, College of Engineering, Defence University, Ethiopia*

Abstract: Bioethanol was produced from Dovyalis Caffra (Kei-apple) which is available in abundance in Ethiopia by using Saccharomyces Cerevisiae, baker's yeast in a batch reactor. Fermentation conditions such as temperature, pH and time were optimized by carrying out experiments at different combinations of conditions. A maximum yield of 5.17%v/v of bioethanol was produced at 32°C and a pH of 5.5 after five days fermentation. Variation of effect of temperature, pH and fermentation time in days was also studied. Material balances were carried out as they are fundamental to the control of processing, particularly in the control of yields of the products. Energy balances carried out to determine the energy requirements of the process, the heating, cooling and power required.
Keywords: Dovyalis Caffra; Saccharomyces Cerevisiae; Bioethanol; Material balance; Energy balance

I. INTRODUCTION

A global attention in preventing greenhouse gases, emanating from fossil fuels, by utilizing biofuels, particularly bioethanol which is a clean burning high octane fuel, produced from fermentation aided by advanced biotechnology processes [1, 2, 3, 4]. One such process is fermentation of Kei-apple which is abundantly available in Ethiopia by Saccharomyces Cerevisiae, baker's yeast [5, 6, 7]. Upscaling of this process of bioethanol produced from the natural resources reduces dependency of many developing countries like Ethiopia on fossil fuel imports thus by saving precious foreign exchange.

The clean burning properties of the ethanol produced will be enhanced by blending it with gasoline and the resulting blend known as gasohol, generally contains 10% ethanol and 90% gasoline (E10) [8, 9,10]. Since ethanol is high octane fuel compared to gasoline and also contains high amount of oxygen (35% by weight) makes the resulting gasohol a clean burning fuel [11]. There are also several automobiles which run on E85 designated gasohol (85% ethanol and 15% gasoline). Gasoline blended with ethanol can also withstand lower temperatures as it is water free alcohol makes it suitable fuel in colder countries.

An estimated growing rate of fuel usage is 10% every year. On an average Ethiopia uses diesel 80,000 cu. M (8 million liter), benzene (gasoline) 1,600cu.m (1.6 million liter), jet fuel 2,500cu.m (2.5 million liter). As per the Ethiopian Petroleum Supply Enterprise (EPSE), Ethiopia would require 2,780,000 metric tons of gasoil, 840,000 metric tons of jet fuel, 494,000 metric tons of gasoline and 83,000 metric tons of fuel oil in 2019. The country's total fuel demand in 2019 is estimated at 4,197,000 metric tons valued at 2.8-3 billion dollars [12].

Ethiopia with a large farm sector, adequate climate and soil conditions allow for both sustainable forestry and agricultural development to satisfy food demand and the sustainable production of renewable biofuels. Large amounts of Kei-apple that are produced by these sectors are given added value as feedstock when using cellulosic ethanol production systems.

The reason Kei-apple being used as feedstock in this study is its abundant availability and which is not being used as food. Hence it will not compete with food consumption and will not require any cultivation land as it grows naturally in wastelands.

Ethanol can be produced from bio-materials in various ways characterized by common steps: hydrolysis of cellulose and hemicellulose to monomeric sugars, fermentation and product recovery. The main differences lie in the hydrolysis phase, which can be performed by dilute acid, concentrated acid or enzymatically [13]. In the present work, acid hydrolysis technique has been considered due to that acids can penetrate lignin without any preliminary pretreatment of biomass, thus breaking down the cellulose and hemicellulose polymers to form individual sugar molecules. Several types of acids, concentrated or diluted, can be used, such as sulphurous, sulphuric, hydrocloric, hydrofluoric, phosphoric, nitric and formic acid [14]. Sulphuric and hydrochloric acids are the most commonly used catalysts for hydrolysis of lignocellulosic biomass [13].

II. MATERIALS AND METHODS

All chemicals used were from reputed brands and used as such without further purification. Double distilled water was used throughout the process.

A. Sample Preparation

The raw material (Kei-apple) was collected from Bishoftu town hall area. Sample preparation process included, manual size reduction (Knife cutting) and grinding after the samples were collected.

B. Chemical Pretreatment

The sample of Kei-apple fruit was washed with 5 % (w/v) potassium permanganate and rinsed with distilled water.

Fig. 1 Collected Kei-apple

Fig. 2 Kei-apple after pretreatment

C. Acid Hydrolysis

The cellulose molecules found in Kei-apple fruit, which are composed of long chains, needed to be broken down to simple sugars, before it's fermentation for alcohol production. Though there are many types of hydrolysis, dilute acid hydrolysis was used as it is an easy and productive process. Diluted sulfuric acid (0.1 %) was added to the non-soluble components and soaked for 24 Hrs. The resulting sample of was then hydrolyzed at a temperature of 110 ^{o}C for 15 min followed by neutralization with 2 M (% w/v) NaoH until the pH has become around 7.

D. Media Preparation

A 125 ml of media was prepared by taking 13 g of Saccharomyces cerevisiae (yeast), 63 g of sucrose and 1.25 g of urea and make up distilled water was added in a 500 ml conical flask. The conical flasks were properly covered with aluminum foils.

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Fig. 3 Media Preparation

E. Sterilization

The fermenter vessel and all the equipment were sterilized by hot water at 121 ºC.

F. pH adjustment

Sodium hydroxide solution was added drop wise to the flask with constant stirring until the pH reaches to 5.5.

G. Fermentation

The fermentation process was started after the initial specific gravity measured by using pycnometer.

The fermentations were carried out under anaerobic condition at temperatures of 25, 28, 32 and 35^{o}C and pH 5.5 for 7 days. During the fermentation process, specific gravity of the sample was noted. Completion of the fermentation was ensured by a constant specific gravity value.

H. Distillation

In the final step of the process, the liquid obtained after fermentation was distilled at 79^{o}C to recover ethanol from the fermented liquid. Distillation was repeated several times until a constant concentration values were obtained to ensure the purity of the ethanol.

Fig. 4 Distillation Process

I. Density Measurements

The ethanol concentrations of the samples collected from the fermented solutions were measured by measuring their densities using a pycnometer [15] at four different temperatures, viz., 25, 28, 32, 35^{o}C.

III. RESULTS AND DISCUSSION

A. Characterization of ethanol produced

Ethanol separated by distillation of fermented liquid was characterized in terms of color, water solubility, specific gravity, refractive index, flame color, boiling point and pH. All values were compared with the standard values from literature and found in a good agreement.

B. *Effect of temperature*

To investigate the effect of temperature on fermentation and amount of bioethanol produced, experiments carried out at different temperatures, viz. 25, 28, 32, 35^{o}C at pH 5.50 for 5 days in a thermostatically controlled water baths gave a maximum yield of bioethanol of 5.17% at 32^{o}C. Yeast will optimally grow temperature in between 30 and 37^{o}C [16]. The results were presented in Table-I and Fig. 5. Above 32^{o}C, the fermentation process was suddenly suppressed. At higher ethanol accumulation, the optimum temperature range becomes narrower. There is also more sensitivity of yeast towards lactic acid and acetic acid at higher temperatures which causes the decrease in ethanol production [17].

TABLE I
Effect of Temperature on Fermentation to Produce Bioethanol at pH 5.50 for 5 Days

Temperature (^{o}C)	Concentration of bioethanol (% v/v)
25	3.08
28	4.77
32	5.17
35	1.61

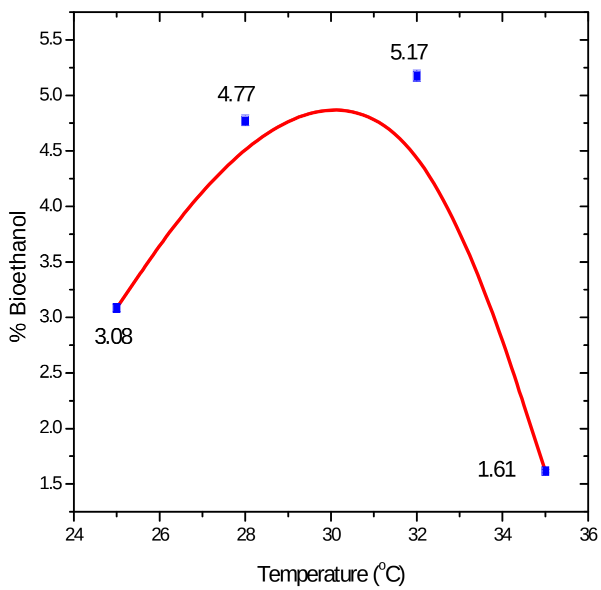

Fig. 5 Effect of temperature on fermentation to produce bioethanol at pH 5.50 for 5 days

C. *Variation of specific gravity with number of days of fermentation at different temperatures*

The steady decrease in specific gravity with progress of fermentation indicates an increase in ethanol production up to 5 days period and after that it achieved almost a constant value. The experiments carried out at four different temperatures viz., 25, 28, 32 and 35^{o}C indicated the similar phenomenon (results were presented in Table-II and Fig. 6).

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TABLE II
Variation of Specific Gravity with days of fermentation at different temperatures

Specific Gravity at	Number of days of fermentation						
	1	2	3	4	5	6	7
25°C	0.99995	0.99810	0.99722	0.99477	0.99245	0.99243	0.99241
28°C	0.99991	0.99900	0.99756	0.99261	0.98829	0.98822	0.98820
32°C	0.99986	0.99825	0.99685	0.99198	0.98782	0.98779	0.98777
35°C	0.99977	0.99897	0.99822	0.99793	0.99688	0.99687	0.99687

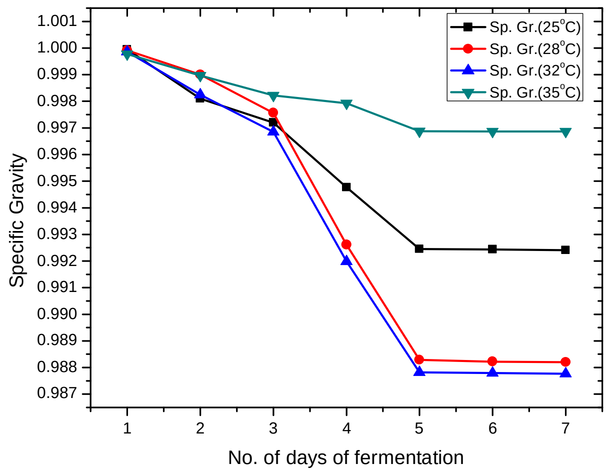

Fig. 6 Variation of specific gravity with number of days of fermentation at different temperatures

D. Variation of pH of the fermentation medium with the progress of the fermentation

Results (Table-III and Fig.7) showed that as the fermentation period increases pH of the medium decreases steadily which can be attributed to the formation of acidic compounds due to microorganism activity in the medium [18]. It has been proved that during the fermentation process acids like acetic, lactic and pyruvic will be formed [19]. Rate of decrease in pH also increases with increase in temperature (25 to 35°C). Yeast survives in a slightly acidic environment with pH ranges from 4 to 6.

TABLE III
Variation of pH with period of fermentation at different temperatures

pH at	Number of days of fermentation						
	1	2	3	4	5	6	7
25°C	5.58	5.54	5.49	5.44	5.37	5.02	4.90
28°C	5.54	5.49	5.42	5.34	5.23	4.93	4.78
32°C	5.52	5.46	5.40	5.30	5.17	4.84	4.68
35°C	5.51	5.44	5.35	5.22	4.98	4.72	4.58

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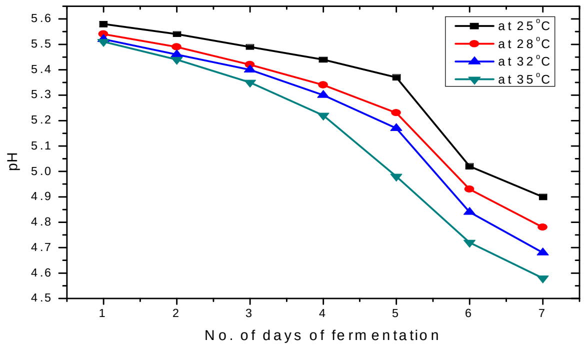


Fig. 7 Variation of pH with progress of fermentation at different temperatures

E. Material Balance

Basis: one day

Production: the plant has the total capacity of ethanol is 5200 L/day of 95% of ethanol.

Amount of pure ethanol in 5200 L ethanol is, 95% of 5200 L = 4940 L

Density of pure ethanol at 20ºC = 0.789 kg/L

So, mass of ethanol at that temperature = density×volume

$= 0.789 kg/L \times 4940L$

= 3897.66 kg

This is 95% of the total distillate; therefore amount of distillate is,

D = mass of water + mass of ethanol.

Where; mass of water = density of water×volume of water

Mass of water = $1 kg/L \times (5\% \times 5200L)$

= 260 kg

Hence, D = 260 kg + 3897.66 kg

= 4,157.66 kg

Changing 95% v/v to w/w,

$$\frac{\frac{\%v/v}{\rho e}\text{ethanol}}{\frac{\%\frac{v}{v}\text{ ethanol}}{\rho e}+\frac{\frac{\%v}{v}\text{ water}}{\rho w}} = \frac{\frac{95}{0.789}}{\frac{95}{0.789}+\frac{5}{1}}$$

= 0.9601

= 96 % w/w

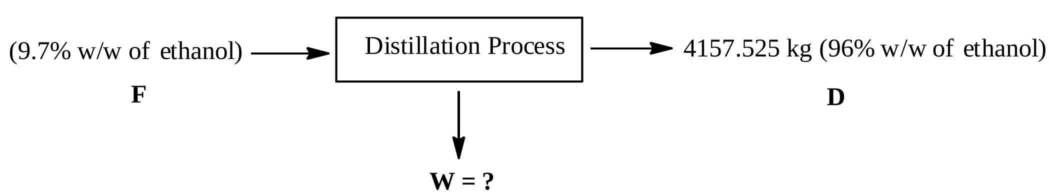
Doing likewise to the feed composition of 7.8 % v/v

We have; $\frac{\frac{7.8}{0.789}}{\frac{7.8}{0.789}+\frac{92.2}{1}} = 0.096839$

= 9.7 % w/w

F. Material Balance On Distillation

Since the bottom product of most distillery contains always negligible amount of ethanol, we assume 0.07 % w/w ethanol in the bottom product.



Applying over all material balance around the distillation column we have,

$$F = D+W$$
$$D = F - W$$
$$F\text{-}W = 4157.525 \text{ kg} \quad \ldots \quad (1)$$

Where D = distillate
F = feed
W = water

Calculate the composition of feed, distillate and water respectively,

$$X_F = \frac{\frac{9.7}{46}}{\frac{9.7}{46}+\frac{90.3}{18}}$$
$$= 0.04$$
$$X_D = \frac{\frac{96}{46}}{\frac{96}{46}+\frac{4}{18}}$$
$$= 0.9$$
$$X_W = \frac{\frac{0.07}{46}}{\frac{0.07}{46}+\frac{99.93}{18}}$$
$$= 0.00027$$

Applying material balance on ethanol for the above distillation column and lettering m_f, m_d and m_w be mass of ethanol in feed, distillate and water (bottom) respectively.

$$F \times X_F = D \times X_D + W \times X_W$$
$$F \times X_F - W \times X_W = D \times X_D$$
$$0.097F = (4157.66 \times 0.96) + 0.007W \quad \ldots \quad (2)$$

Solving equation (1) and (2) simultaneously we have,

W = 39867.34 kg
F = 44024.865 kg
D = 4157.525 kg

G. Mass Balance On Fermenter

The reaction in the fermenter is

$$C_{12}H_{22}O_{11} + H_2O \xrightarrow{\text{hydrolysis}} C_6H_{12}O_6 + C_6H_{12}O_6$$

Sucrose → Glucose, Fructose

$$C_6H_{12}O_6 \xrightarrow{\text{yeast}} 2CO_2 + 2C_2H_5OH \qquad (\Delta H = -31.2 \text{ kcal})$$

180 g/mol → 92 g/mol

Accounting for ethanol loss in distillation column bottom product to be 10% of total, therefore, mass of pure ethanol out of fermenter is

$$= \frac{4157.525}{0.9}$$
$$= 4619.47\ kg$$

If 92 g/mol of pure ethanol requires 180 g/mol of glucose, producing 4619.47 kg pure ethanol will require,

$$\frac{180 \times 4619.47}{92}$$

= 9038.09 kg of glucose.

Assuming 94%conversion of glucose, actual glucose required is,

$$\frac{9038.09}{0.94}$$

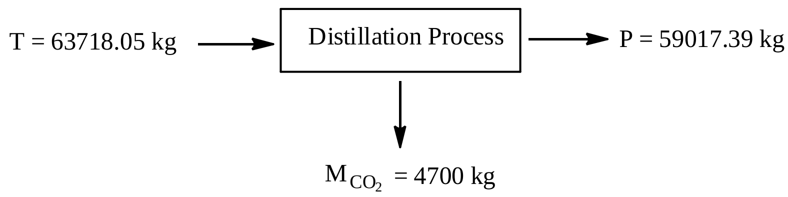
= 9614.99 kg of glucose

From the reaction of 180 g/mol glucose, we get 88 g/mol of CO_2

9614.99 kg of glucose will give,

$$\frac{88 \times 9614.99}{180}$$

$= 4700.66\ kg\ \ of\ CO_2$



$$T = P + M_{CO_2}$$

Where T = Total sample in the fermenter

M_{CO_2} = mass of CO_2

P = fermented sample

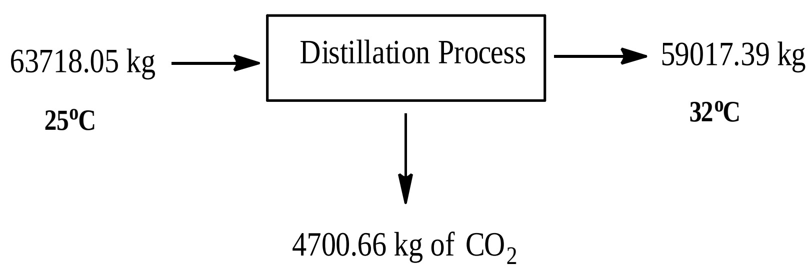
T = 4700.66 kg of CO_2 + P

63718.03 = 4700.66 kg + P

P = 59017.39 kg

H. Energy Balance

Energy balance on fermenter



Fermentation is an exothermic reaction heat will be generated inside the fermenter and the outlet temperature is 32°C.
Inlet temperature is 25°C.
From the reaction,

$$\underset{180\ g/mol}{C_6H_{12}O_6} \xrightarrow{\text{yeast}} 2CO_2 + \underset{92\ g/mol}{2C_2H_5OH} \qquad (\Delta H = -31.2\ kcal)$$

To produce 92 kg of ethanol 31.2 kcal heat is released.
How about producing 4619.47 kg ethanol

$$\frac{4619.47 \times 31.2}{92} = 1566.61 \text{ kcal is realesed}$$

Cooling water must be required to keep temperature in the fermenter from not rising above 35° so that the yeast is not killed.
Heat balance becomes,
Heat taken by water per day = heat liberated by fermenter per day

$$Q_W = Q_F = 1566.6115 kcal$$

$$= M_w\, Cp_w\, \Delta T_w$$

Fermenter temperature should be maintained below 35°C is for cooling temperature.
Mass of water to remove that much heat is

$$= M_W \times {}_{0.9989}\, (32°C - 25°C)$$

$= 1566.61$ kcal (because Cp_W at 25°C = 0.9989 kcal/kg°C)

$M_W = 224.046$ kg

I. Energy Balance On Distillation Column

C_p of water = 1.0029 kcal/kg°C

V_{BCp} of ethanol = 0.623 kcal/ kg°C

C_p of feed = $X_w C_{pw} + X_e C_{pe}$ = ((1-0.097)×1.0029 + (0.097×0.623))

= 0.966 kcal/kg°C

C_p of distillate = $X_w C_{pw} + X_e Cp_e$

= ((1- 0.96)×1.0029+ (0.96×0.623))

= 0.6381 kcal/kg°C

C_p of bottom product = $X_w C_{pw} + X_e Cp_e$

= ((1-0.0007)×1.0029 + (0.0007×0.623))

= 1.0026 kcal/kg°C

Reference temperature =25°C = 298°K

Heat output by distillate= $m_d C_{pd} \Delta T_d + m_d H_d$

= ((41577.525×0.96)(85°C-25°C)×0.6381+(0.96×4157.525×204.26)+(1-0.96)×4157.525×540)

$\Delta H_d = 11.2456 \times 10^5$ kcal

Heat input due to feed = $M_f C_{pf} \Delta T_f$

= 47038.69 × 0.966 × (91.57-25)

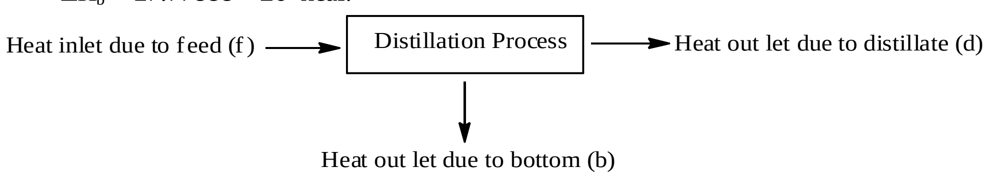
Where 91.57 is bubble point temperature of feed

$\Delta H_f = 30.24899 \times 10^5$ kcal.

Heat output to bottom product = $M_b C_{pb} \Delta T_b$

= 42641.169×1.0026×(90°C-25°C)

$\Delta H_b = 27.77888 \times 10^5$ kcal.



So, the total heat input to the distillation column

$= \Delta H_b + \Delta H_d - \Delta H_f$

= ((27.77888+11.05476) – 30.248) =8.5847× 10^5 kcal

IV. CONCLUSIONS

The main objective of this study was to determine optimal anaerobic fermentation conditions for bioethanol production from Dovyalis Caffra (kei-apple) using yeast in a batch reactor through experimental approach. The following conclusions were drawn from the study:

1) A maximum amount of bioethanol, 5.17 %v/v was obtained at optimum conditions, 32°C and 5.5 pH.
2) Variation of bioethanol production with change in temperature was carried out and found that 32°C as optimum temperature
3) A study of variation of specific gravity with progress of fermentation at different temperatures revealed a steady decrease in specific gravity with progress of fermentation
4) A study of variation of pH with progress of fermentation concluded that a steady decrease in pH as fermentation progresses.
5) Material balances were carried out as they are fundamental to the control of processing, particularly in the control of yields of the products.
6) Energy balances carried out to determine the energy requirements of the process, the heating, cooling and power required.

V. ACKNOWLEDGEMENT

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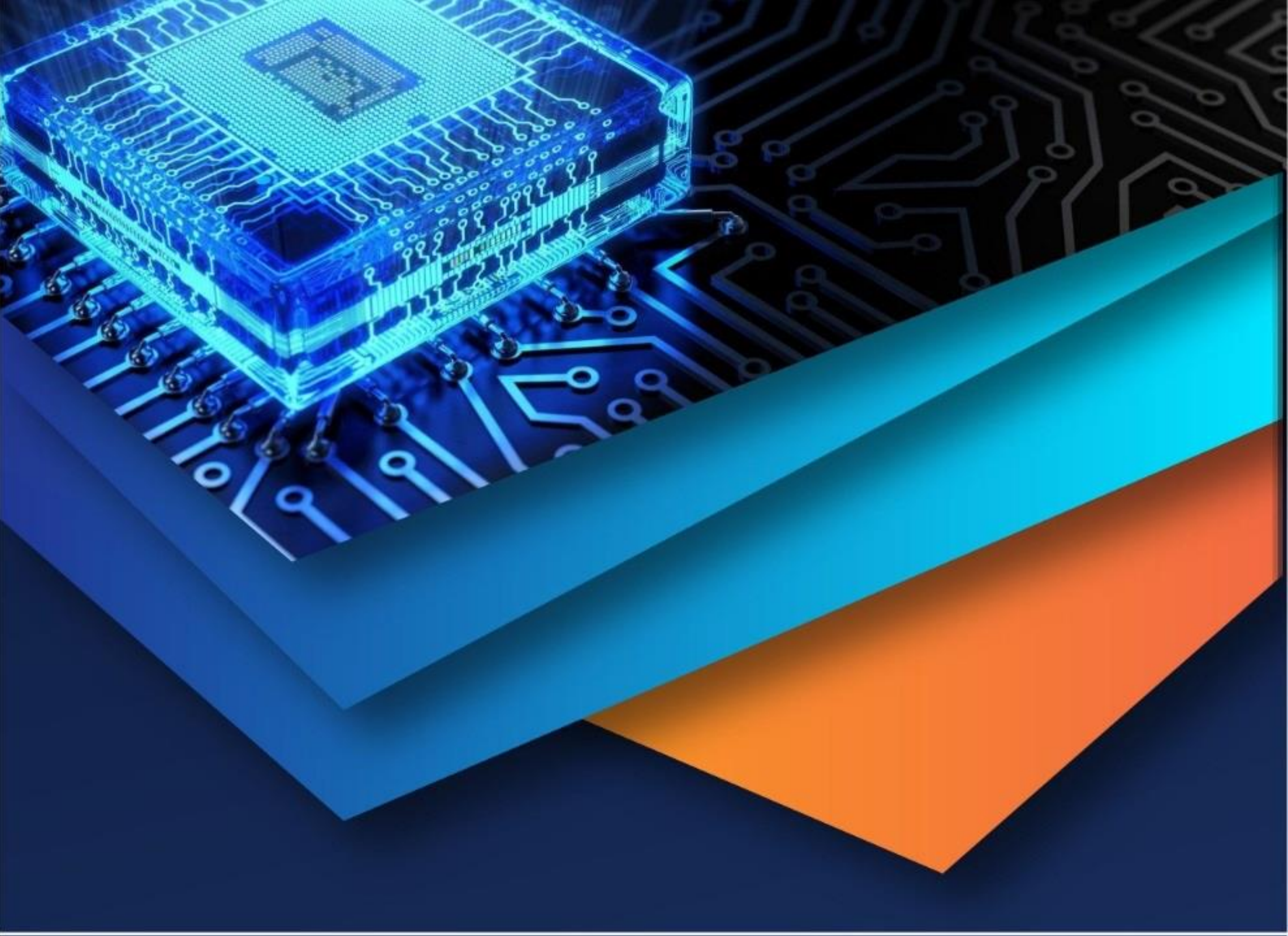

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