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Isolation and Screening of Amylolytic and Pectinolytic Bacteria from Indigenously Prepared Bioenzyme and its Application for Pond Water Treatment

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Abstract: ***Bioenzyme is produced by the fermentation process of citrus fruit peels, water and jaggery. It is an effective alternative to harsh chemicals such are phenyl, detergent and bleach which are commonly used as a cleaning agent. Excessive usage of harmful chemical product causing degradation of our ecosystem and the objective is to treat pond water using bioenzyme because water contain many chemical from household detergents. The present work comprises screening and isolation of amylolytic and pectinolytic bacteria from indigenously prepared bioenzyme. The starch agar plate and PSA media was used to screen bacteria. Morphological and biochemical characteristics of the screened isolate were tentative identified as Basillus sp. and determined their enzyme activity by DNS method. The enzyme activity of both amylase and pectinase producing bacteria are ranged from 229.5 µmole/min to 936.42 µmole/min and analysis of parameter like BOD for pond water treatment. Different conc. of bioenzyme used for treatment. After treating pond water with it, there was substantial change in BOD value from 44mg/lit to 12mg/lit. It means BOD levels reached permissible level after the treatment.***
Keywords: Bioenzyme, Amylolytic, Pectinolytic, Biological oxygen demand, Pond water

I. INTRODUCTION

The Enzyme are biological catalyst and also called as biocatalyst that speed up reaction in the living organism and can be pulled out from cell and then used to catalyst a wide range of commercially important processes. Such as production of sweetening agent, modification of antibiotic also used in washing powder and various cleaning agents. [1]

A. Bioenzyme

Bioenzymes are organic compound produced by fermentation of fruit peels in the presence of water and jaggery. These organic substance are capable of the breaking down of chemical and other organic waste thus helping us in removing stanis odour, getting rid of the other harmful microbes.

Bioenzyme is an effective alternative to harsh chemical like detergents which are generally used in households as cleaner for toilet, bathrooms and other surface. Bioenzyme helps to reduce certain waste materials and turn into a useful substance to the society is economically and cheaply available and the end product can be completely useful. [2] [3]

B. Amylase

Amylase is an extracellular, glycoside enzyme that catalyzes the hydrolysis of complex sugar such as starch into subsequent simple sugar molecules. Amylase enzyme can be produced by microorganism especially amylolytic microbes such as *Bacillus subtillis, B. aureus, B. azotoformans* etc.[4]

C. Pectinase

Pectinase there are various enzymes which are produced naturally by living organisms and more specifically by microorganism which are used for enzyme production.

Pectinase are heterogenous enzyme that hydrolase pectin substance and mostly present in bacteria fungi and higher plants. Pectinolytic bacteria are *Erwinia sp., Pseudomonas fluorescens, B. licheniformis, B. cereus.* [5][6]

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II. MATERIALS & METHODS

We first have to make bioenzyme in this experiment from which amylolytic and pectinolytic bacteria will be isolated and then use that bioenzyme as a cleaner in pond water treatment.

A. Bioenzyme Preparation

We were prepared the bioenzyme by taking the 1:3:10 ratio. In this ratio the 1 part of jaggery, 3 parts of citrus peels and 10 parts of water. After that, we kept it for fermentation for 3 months in an airtight container. In the process of 3 months, in the first month gases generated which were allowed to evolved and later two months kept in the airtight container for further fermentation of the citrus peels. After that the enzyme will be ready for use. [7]

B. Isolation & Screening of Amylolytic and Pectinolytic Bacteria from Bioenzyme

Isolation of amylolytic bacteria from bioenzymes was done by streak plate method through starch agar media and their primary and secondary screening were also done by streak plate method in starch agar plate. After 24 hrs. incubation 37ºC, iodine solution was flooded in the plates then observed the clear zone obtained around the bacterial colony.[8]

Same as, Isolation of pectinolytic bacteria from bioenzyme was done by streak plate method in PSA (Pectinase Screening Agar) media and their primary screening was also done by Streak plate method in PSA media and secondary screening was done by well method in PSA media. After 24 hrs. incubation at 37ºC of this petriplate, iodine solution was flooded in the plate and observed the clear zone around the bacterial colonies.[9][10]

C. Identification of Amylolytic and Pectinolytic Bacteria

The Bacterial isolates with prominent zones of clearance was processes for the determination of morphology, Gram characteristic, Citrate utilization, Methyl Red test, VP test , Indole test, Catalase test and Oxidase test.[11][12]

D. Production of Crude Amylase and Pectinase Enzyme

Isolates with highest clear zone in starch agar media and PSA media were incubate in starch broth and pectinase broth respectively at 37ºC for 3 days and after incubation the media from each flasks were centrifuged at 10000 rpm for 15min. And crude amylase and pectinase enzyme were obtained.[13]

E. Determination of Enzyme Activity

Enzyme activity was measured by the estimation of the amount of product produced through the DNS method. Taken 1 test tube and added reaction mixture which consisted for the determination of amylase activity, 1% substrate(0.1gm starch dissolved in 100ml of distilled water) 1ml and 1ml of crude enzyme and for the determination of pectinase activity reaction mixture which consisted of substrate 1%(0.1gm pectin dissolved in 100ml of Distilled water) 1ml and 1ml of crude enzyme. These mixture were incubated at 37ºC for 30min in incubator. Later 1ml of DNS reagent was added to the test tube and kept in boiling water bath for 10min, solution turned to reddish colour. A blank was also prepared in the same way and used distilled water in place of enzyme for blank. The absorbance was read at 550nm using spectrophotometer and a graph was drawn by plotting absorbance against conc. [14]

Estimate the conc. of reducing sugar in crude enzyme from the slope of graph and calculate the enzyme activity from formula. [15]

$$\text{Enzyme activity (µmole/min)} = \frac{\text{µg of product released} \times 1000}{\text{Mw. of Product} \times \text{incubation time in min.}}$$

F. Application of Bioenzyme for Pond Water Treatment

Use of bioenzyme is an effective method of treatment of pond water. To investigate the effectiveness of bioenzyme application as an alternative method in pond water treatment by measuring BOD before and after application of enzyme specially selected bacteria release enzyme that liquefy and then literally accelerate the digestion process of organic content present in pond water.[16]

To estimation of DO is the most important step in BOD calculation of water is usually estimated by winkler's method which is titrimetric method and calculate the amount of DO by using following formula- [17][18]

$$DO = 8 \times \frac{1000 \times N \times v}{V} \qquad BOD = D_1 - D_2$$

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Where, V = Volume of Water Sample use for Titration
N = Normality of Titrate (0.025N)
v = Amount of Sodium thiosulfate
8 = Constant value of Equivalent
BOD = Biological oxygen demand
D_1 = Initial dissolved oxygen(DO) , D_2 = Final dissolved oxygen

III. RESULT AND DISCUSSION

Bioenzyme was produced by the fermentation process. The solution was filtered after 3 months to obtain bioenzyme. That obtained bioenzyme solution was light brownish yellow coloured. Total 830ml of bioenzyme solution was prepared. From the isolation and screening of amylolytic bacteria there were only single type of bacterial colonies grown on the starch agar plate after incubation and clear zone was observed after treat with gram's iodine solution and their diameter was 15mm. and from the isolation and screening of pectinolytic bacteria, there was also only single type of bacterial colonies grown on the PSA media plate and their observed clear zone diameter was 20mm. The identification of the selected bacterial strains were done on the basis of morphological and biological characteristics. On the basis of morphology and biochemical tests, the selected amylolytic bacterial strain was identified as *Bacillus sp*. and pectinolytic bacterial strain was also identified as *Bacillus sp.*. The details of biochemical characteristics of pectinolytic and amylolytic are given in table:2

The Determined amylase and pectinase enzyme activity by DNS method in which reducing sugar were measured by adding 3,5 Dnitrosalicylic acid reagent, using maltose, galacturonic acid respectively as standard and the enzyme activity of crude amylase, pectinase enzyme were calculated as 229.5µmole/min, 936.42µmole/min respectively.

Application: Effect of Bioenzyme in the Treatment of Pond Water

This study is carried out on pond water, upon treating pond water with bioenzyme there was significant change in BOD value of the water. Characteristics of pond water before and after treatment with bioenzyme were analyzed and indicated in table5. It was proven that bioenzyme has a promising effect in the treating of pond water as the reduction in level of BOD 44mg/lit to 12mg/lit.

Table No. 1 Bioenzyme Production

Jaggery	Fruit Peels	Water	Bio-Enzyme
90g	270gm	900ml	830ml BrowncolorCitrus odour

Table No. 2 Identification of Amylolytic and Pectinolytic Isolates

S. NO.	Tests	Amylolytic Isolates	Pectinolytic Isolates
1.	Morphological Test: Gram Staining	Gram Positive	Gram Positive
2.	Biochemical Test:		
a.	Catalase Test	Positive	Positive
b.	Oxidase Test	Negative	Negative
c.	Indole Test	Negative	Negative
d.	MR Test	Negative	Negative
e.	VP Test	Positive	Positive
f.	Citrate Utilization Test	Positive	Positive

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Table No.3 Standard Series of Maltose (For Amylase activity)

S. No.	Volume of Maltose	Volume of Distilled Water	Concentration Of Maltose	DNS Reagent	Incubation	O.D
1.	0.1	0.9	100	1ml	Boil	0.22
2.	0.2	0.8	200	1ml	In	0.26
3.	0.3	0.7	300	1ml	Water	0.35
4.	0.4	0.6	400	1ml	Bath	0.39
5.	0.5	0.5	500	1ml	For	0.43
6.	0.6	0.4	600	1ml	10 mins.	0.48
7.	0.7	0.3	700	1ml		0.53
8.	0.8	0.2	800	1ml		0.62
9.	0.9	0.1	900	1ml		0.75
10.	1.0	00	1000	1ml		0.81
11.	Unknown			1ml		0.55

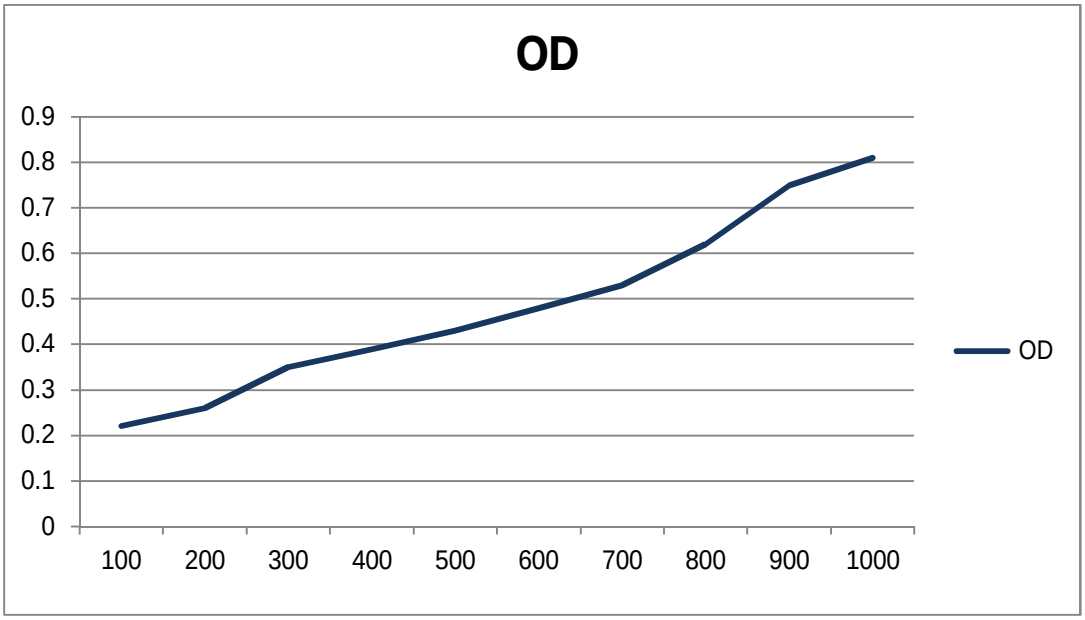


Fig. 1 Graph Between Maltose conc. and their OD (For Amylase)

Calculate the slope from standard graph of maltose for Amylase activity:

$$\text{Slope} = \frac{Y_2 - Y_1}{X_2 - X_1}$$

Where ,

Y= OD at 540nm

X= Conc. of Maltose

$$\text{Slope} = \frac{0.62 - 0.48}{800 - 600} = 0.0007$$

Concentration of maltose(µg/ml) = O.D/ Slope

= 0.55/ 0.0007

= 785µg/ml

$$\text{Enzyme activity} = \frac{\mu g \text{ of product released} \times 1000}{\text{Molecular weight of product} \times \text{incubation time}}$$

$$= \frac{785 \times 1000}{342 \times 10}$$

Amylase activity = 229.5 µmole/min

Table No. 4 Standard series of Galacturonic acid (for Pectinase activity)

S. NO.	Vol. of Galacturonic acid(ml)	Vol. of DW(ml)	Conc. Of Galacturonic acid(µg)	DNSA	Incuba--tion	OD Value
1.	0.1	0.9	100	1ml	Boil	1.05
2.	0.2	0.8	200	1ml	In	1.11
3.	0.3	0.7	300	1ml	Water	1.17
4.	0.4	0.6	400	1ml	Bath	1.24
5.	0.5	0.5	500	1ml	For	1.31
6.	0.6	0.4	600	1ml	10	1.39
7.	0.7	0.3	700	1ml	mins	1.44
8.	0.8	0.2	800	1ml		1.51
9.	0.9	0.1	900	1ml		1.57
10.	1.0	0.0	1000	1ml		1.63
11.	Unknown			1ml		1.09

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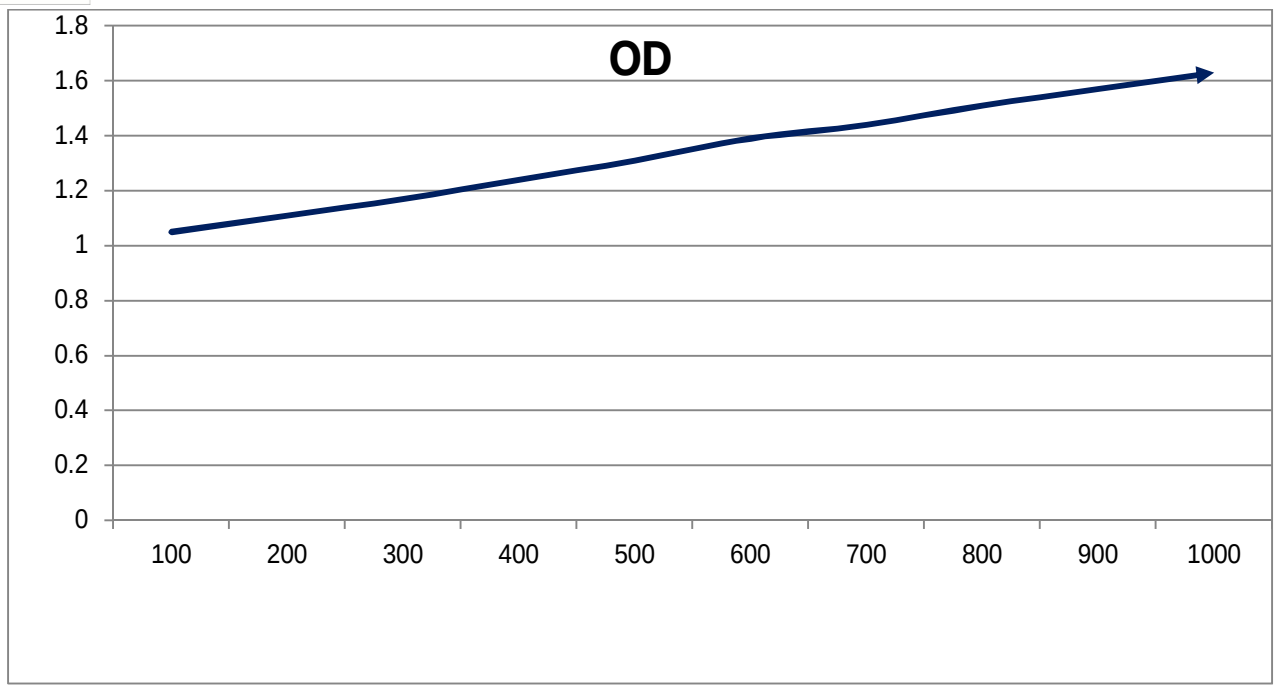


Fig. 2 Graph between OD in Y axis and Galcturonic conc. in X axis(For Pectinase)

Calculate Pectinase Enzyme Activity:

$$\text{Slope} = \frac{1.57 - 1.44}{900-700}$$

$$= 0.13/200 = 0.0006$$

Concentration of Galacturonic acid = OD/ Slope

$$= 1.09/0.0006 = 1816.6\ \mu g/ml$$

$$\text{Enzyme Activity} = \frac{1816.6 \times 1000}{194 \times 10} = 936.42 \mu mole/min$$

Calculated Pectinase activity = 936.42µmole/min

Table No. 5 Determination of BOD

S. No.	Criteria	BOD
a)	Before Bio-enzyme Treatment	44
b)	After Bio-enzyme Treatment	
i.	1st BOD bottle:5ml Bio-enzyme	38
ii.	2nd BOD bottle:10ml Bio-enzyme	32
iii.	3rdBOD bottle: 15ml Bio-enzyme	20
iv.	4th BOD bottle: 20ml Bio-enzyme	12

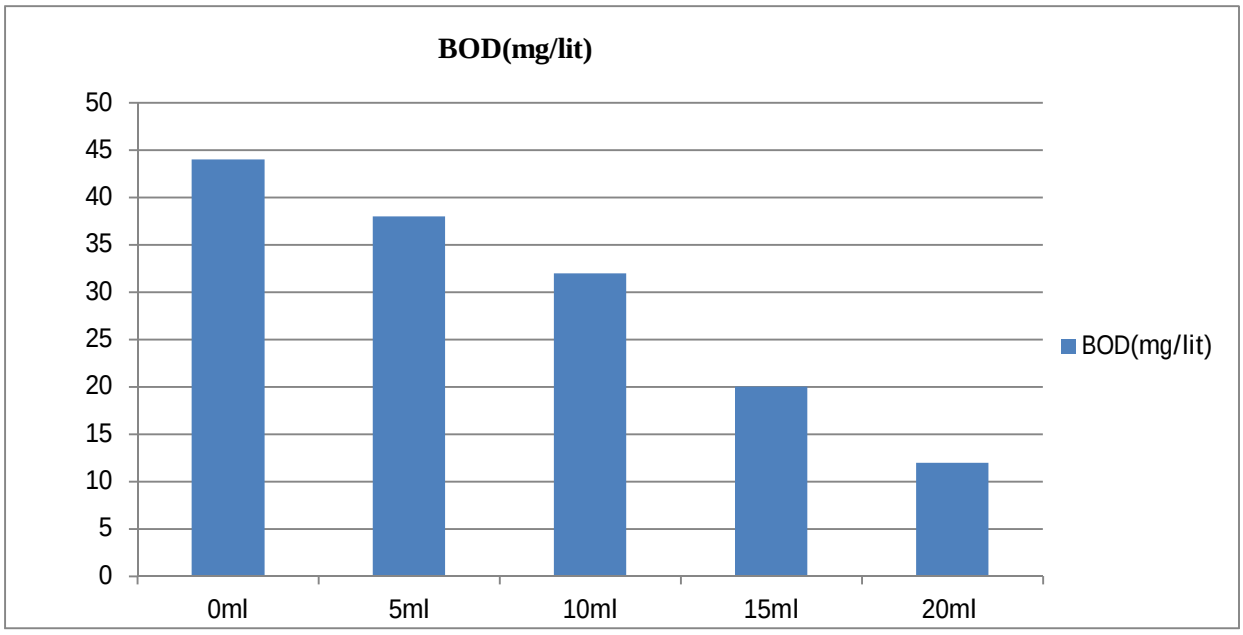


Fig. 3 Graph between BOD and Bioenzyme treatmen



Fig. 4 Bioenzyme Production



Fig. 5 Cultured plate of Starch agar media

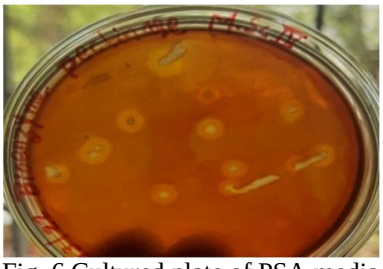


Fig. 6 Cultured plate of PSA media

IV. CONCLUSION

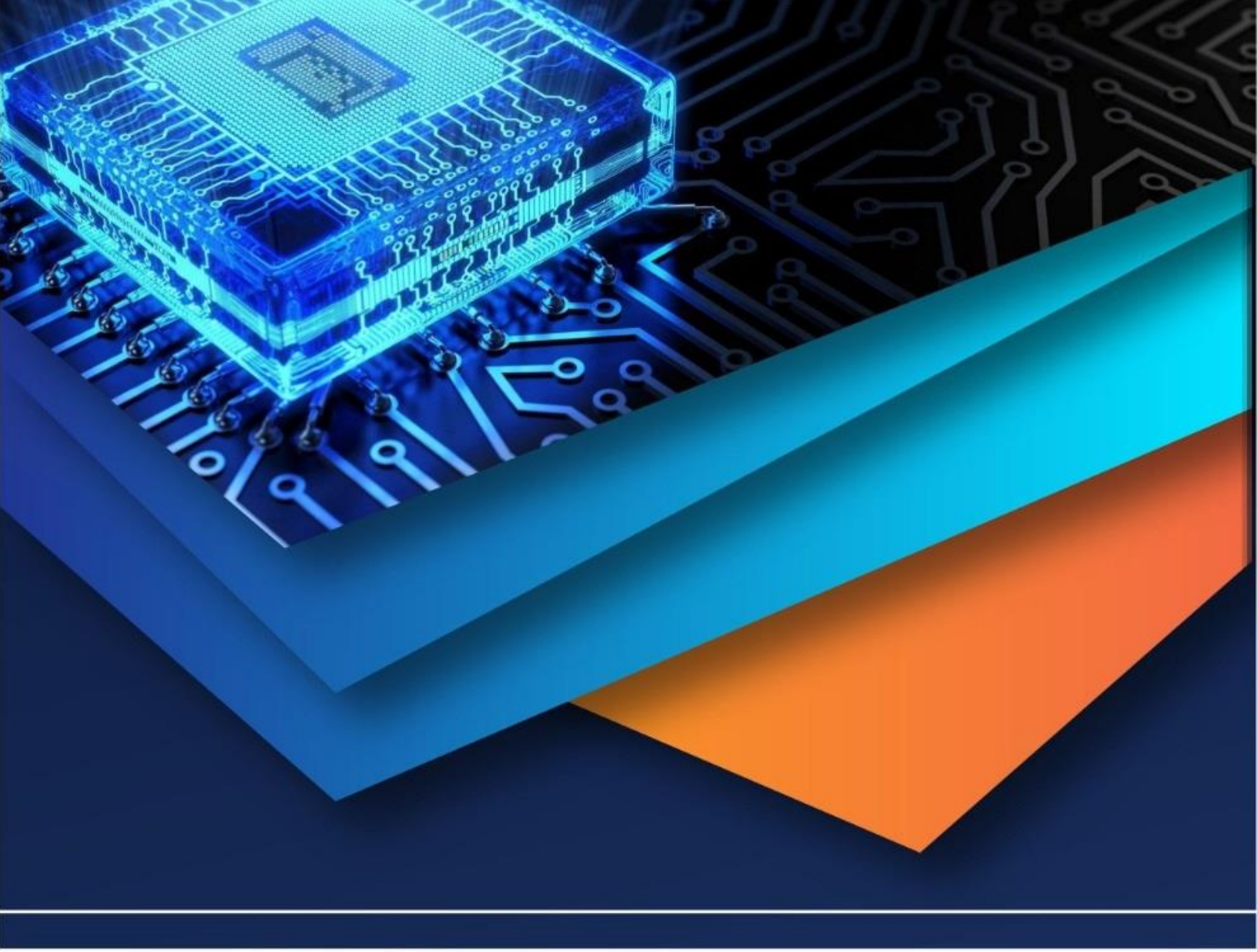
The whole study concluded that the enzymes are biological catalysts. In the present study bioenzyme produced using citrus fruit waste after potential solution to treat pond water in ecofriendly way. Result show that bioenzyme accelerate the digestion process of organic content present in pond water. This show that the water to be cleaner after the treatment. Bioenzyme is a multipurpose solution for domestic and agriculture application and it is organic it won't have any side effects. Compare to other method this can be an easy and cost effective method and reduce the use of synthetic chemical that are toxic to human health and the environment. "Conversion of Wastes into Value Added Products is Potentially Profitable besides Creating a Clean Environment."

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