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Comparative Studies of Invertase Enzyme from Low Cost Agricultural Waste Substrates By Saccharomyces CerevisiaeIsolated From Grape Juice

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Abstract:*The official name for invertase is β- fructofuranosidase (EC.3.2.1.26), which implies that the reaction catalyzed by the enzyme, is the hydrolysis of the terminal non-reducing β-fructofuranoside residues in β-fructofuranosides.In this present study screen the industrially important yeast Saccharomyces cerevisiaefrom grape juice sample using serial dilution and yeast peptone dextrose agar plating technique. The isolate was checked for the invertase enzyme production by using different low cost agricultural waste substrates like Ricebran,Wheatbran,Ground nut cake flakes and Sucrose.Invertase was characterized by protein and carbohydrate estimations and enzyme assay. The partial purification of this enzyme was studied by ammonium sulphate precipitation, membrane Dialysis. The results showed in this study about 43.5 U/ml for Rice bran, 72.5 U/ml for Wheat bran, 63.3U/ml for Ground nut cake flakes and 83.5U/ml for Sucrose.*

Keywords: ***Saccharomyces cerevisiae, yeast, yeast peptone dextrose agar, Ammonium sulphate precipitation, protein, carbohydrate****.*

I. INTRODUCTION

Invertase is a digestive enzyme that separates Sucrose into its main components, fructose and glucose. Usually derived from saccharomyces cerevisiae and afterwards purified to be utilized alone or within a multi enzyme formula, invertase is mixed up with some other carbohydrates to enhance overall starch and sugar digestion.

In 1877, German physiologist Wilhelm Kuhne (1837-1900) first used the term enzyme, which comes from Greek “leavened”, to describe this process. The enzyme was used later to refer to nonliving substances such as pepsin, and the word ferment was used to refer the chemical activity produced by living organisms. Since this type of enzyme is high biotechnological potential β-D-fructofuranosidase, can be produced by many organisms, especially microorganisms like bacteria, yeast and filamentous fungi therefore it are produced in different formed by either intracellular or extracellular invertase[4] such as Neurosporacrassa, Candida utilis, Fusariumoxyporphytophtorameganosperma, Aspergillusniger, Saccharomyces cerevisiae, schizosaccharomycespombe and schwanniomyces occidental[14].

The ability of invertase to hydrolyze the connection between glucose and fructose makes the enzyme an important part of the digestive process. Additionally known as beta- fructofuranosidase, invertase isconsidered an essential enzyme that can easily help people digest sugars. It can be found in yeast sources and pollen as it has a key role not just in the digestive process but also in the overall prevention of human diseases. Invertase can also slow down the anti- aging progression and it can help people enjoy physical rejuvenation.

Invertase is one of the beneficial enzyme that provides many products for industrial purpose such as pharmaceutical, food and etc. it is due to its utilization, its function to hydrolyse the Sucrose into equimolar mixtures of glucose and fructose at a concentration lower than 10 % Sucrose[6]. Therefore, this enzyme has attract researcher attention to study on any potential method of production that serves high effectiveness production method of microbial invertase in which the acid hydrolysis process but with low conversions efficiency which is 65-70% [8].

It is one of the earliest enzyme discovered by researcher which is isolated in the second half of the 19th century and it is become a valuable enzyme due to its own function which is produced “invert sugar” in ratio of mixture 1:1 of mixture of dextrorotatory D-glucose and levorotatory D-fructose. Therefore, there is crucial to recognize the structure and function of invertase.

This enzyme was not specifically defined in its structure until now. Researchers has proposed that GH32 yeast invertase structure has not been reported until now, a remarkable fact when taking into account that yeast invertase have been described as

multimeric[3]. Moreover, this research also proposed that the basic structural unit of intracellular and extracellular invertase is in dimer form as shown by electron microscopy but can be transformed into larger oligomers structure upon mannose binding[3]

In fact, these enzymes have different structure of isoforms at different optimum p^H but this different structure of enzyme were not reported to have specific function. It was only stated to have function as to control the entry of Sucrose into different utilization [1]. According toAlegre et al.year 2009, for acidic forms of invertase appear to have cell wall or vacuolar localization and structurally related to yeast and bacterial invertase. However, in neutral and alkaline forms were found in the cytosol [16].

Further review lead to increase in understanding of invertase function. It is a member of GH32 family of glycoside hydrolases, which include more than 370 enzymes of vegetable and microbial origin.[6] .It is function to hydrolyse the 1, 4-glycosidic bonds of Sucrose and eventually formed equimolar mixtures of glucose and fructose that referred as invert sugar [11]. It is a type of enzyme which is used for the inversions of Sucrose in the preparation of invert sugar and high fructose syrup [15].

Since it is naturally exist, therefore many researchers has been attracted to conduct a study on the contribution from this enzyme to the world nowadays. it represents the chemical structure of Sucrose hydrolysis reaction catalyzed by invertase one of the most extensively recycled enzymes in food engineering, especially in the preparation of confectionaries, food industries and in pharmaceuticals[2].Invertase is used for the invertion of Sucrose in the preparation of invert sugar and high fructose syrup (HFS).The present study trend is the utilization of waste material for production of Yeasts invertase enzyme by natural isolates which boosts up high economic returns in many industries.

In this present study deals the production and partial purification of this economically important enzyme Invertase by using economical agro waste materials to satisfy the need of this enzyme.

II. MATERIALS AND METHODS

A. *Isolation of yeast culture from grape fruitextract.*

Grapes collected from local market, Tiruchirappallidistrict. Grape fruits crushed using mortar and pestle extract filtered and filtrate serially diluted and plated on yeast peptone dextrose agar plates both by pour and spread plate methods. The Isolates were identified on the basis of their morphological and biochemical characteristics include Gram's staining, Lacto phenol cotton blue staining, Glucose, Sucrose, Lactose, Mannitol, Maltose. Single germinating spores were picked from the mixed culture and sub cultured. A pure culture was produced by repeated sub culturing. The purified cultures were then transferred to YPD medium and sub cultured which was then stored at 4^0C until use.

B. *Quantitative estimation of protein by Lowry's Method*

It is most commonly used method for determination of protein in cell free extracts because of its high sensitivity and qualities as low as 20mg of protein can be measured. The CO-NH (peptide bond) in polypeptide chain reacts with copper sulphate in an alkaline medium give a blue coloured complex. In addition tyrosine and tryptophan residues of protein complex causes reduction of the phosphate bond. Phosphomolybolate and phosphotungstate components of folinceocalteau reagents to give bluish products which contribute towards enhancing the sensitivity of this method. It is however, important to remember the several compounds like EDTA, Tris, CHO, NH_4^+, K^+, Mg^{++} ions thiol reagent, phenol etc… interfere with the colour development and it should be ensured that such substances are not present in sample preparation.Pipette out aliquots of egg albumin working standard 0.1-0.5ml in different test and makeup in different final volume to 1ml with water.1ml of water serves as "Blank". To all test tubes including 1ml of diluted of the tubes solutions and 5ml of alkaline copper sulphate and 0.5ml of diluted Folinciocalteau reagent rapidly. Mixed immediately after 30mintues read optical at 620nm [9].

C. *Test for carbohydrates*

1) *(Barfoed's test):*To 2ml of the extract, add 1ml of Barfoed's reagent and boil. Reddish brown indicate the presence of carbohydrates.
2) *(Benedict's test):*To 5 ml Benedict's reagent, add 1 ml of extract solution and for 2 minutes and cool. Formation of red precipitate shows the presence of sugar.
3) *Effect of Agro waste substrates on invertaseenzyme production:*Production media was prepared as above with optimized concentrations of Sucrose-5g, Yeast extract-5g, Ammonium sulphate 0.25g, Megnesiumsulphate 0.018g, potassium dihydrogen phosphate 0.87g.using this basal media 5%Wheat bran, 5%Rice bran, 5% Ground nut cake flakes and 5% of commercial Sucrose substrates were added to the production medium and the culture was inoculated. The enzyme carried assay was carried out.

D. Enzyme extraction

The enzyme extraction was carried out by centrifuging the 24hours old culture at 10,000 rpm for 10 minutes. The supernatant was considered as crude enzyme solution and stored at 4^0C.

E. Ammonium per Sulphate Precipitation

A volume of crude enzymes were taken separately, added slowly the required quantity of ammonium per sulphate. The addition of ammonium per sulphate was done under constant stirring at 4^0C for 30 minutes and then stirring was continued for another 30 minutes and then allowed for settlement for 3hrs at 4^0C. The precipitated proteins were separated by centrifugation at 8000 rpm at 4^0C for 20 minutes. The separated proteins were then dissolved in minimum amount of 0.05M citrate buffer (p^H - 5) and refrigerated for further analysis.

F. Membrane dialysis

Membrane dialysis also known as Visking tubing, is a type of semi-permeable membrane tubing which is used in separation techniques, that facilitates the removal or exchange of small molecules from macromolecules in solution based on differential diffusion. The crude protein sample filled inside the membrane pouch and the membrane bag hanged inside the buffer solution. Periodical changes of buffer solution were done.

G. Enzyme assay

Into each of a series of 30 ml of test tubes, pipette, in quadruplicate, 1.4 ml of water, 0.5 ml of acetate buffer and 0.1 ml of diluted enzyme. Equilibrate the tubes in a 30^0 water bath. Add 1ml of 0.3 M Sucrose solution to 3 of the 4 tubes. Uses the fourth tube as an enzyme blank, adding 2 ml of DNS blank using 0.1 ml of water in place o diluted enzyme. Reaction and Measurement. Read the respective contents of invert sugar from the calibration curve.[5]

H. Calculation

Activity for liquids (units/ minute/ml) = $(C_S.C_B) \times 10 \times$dilution $\times$ S.G.

Where,
Cs = content of invert sugar in sample solution (micromoles)
C_B= content of invert sugar in enzyme blank solution (micromoles)
W = weight of sample (g)
S.G = specific gravity of sample (g/m)

III. RESULT AND DISCUSSION

A. Morphological Identification

The Staining and Microscopic observation was done forSaccharomycescerevisiaeis Gram positive organism. It is single celled classified as members of the fungus kingdom. It size range from 3-4 µm in diameter, measured by Micrometry method. It is grows well on YPD Agar medium it produce smooth moist and glistering colonies dull cream in colour. Lactophenol cotton blue staining the cells wereSpherical, cylindrical with rounded ends

B. Test for Carbohydrates

The result of qualitative analysis of reducing sugar, partially purified supernatant sample of Wheat bran, Rice brawn, Ground nut cake flakes flakes and Sucroseshows the presence of reducing sugar were present in table 1.

C. Protein analysis – Lowry's Method

The results of protein analysis of lowery's method were presented as graphical representation the amount of protein present in the Wheat bran, Rice brawn, Ground nut cake flakes flakes and Sucrose in the substrates. In Wheat bran the amount of protein presented in the calculated value is 1.6 gm,inRice bran the value is 1.5gm,in Ground nut cake flakes flakes the value is 1.3gm and the Sucrose showed 1.4gm.From this above results Wheat bran give better sufficient enzyme amount and hence Wheat bran consider as a economically valuable substrates. (Figure1)

D. Enzyme Assay

Substrate utilization (Wheat bran, Rice bran, Ground nut cake flakesand Sucrose) by saccharomyces cerevisiae for the production of invertase enzyme in fermentation were studied. In optimized cultivation conditions production of invertase assay value was 72.4 U/ml for Wheat bran, Rice bran showed43.5 U/ml,Ground nut cake flakes showed63.3 U/ml and Sucrose showed 83.5 U/ml. (Table 2)

invertaseenzyme produced from various type of microorganisms includes fungi, yeast and bacteria. The most type of microorganism that can produced this enzyme is fungi type such as Aspergillusniger [2],Aspergillusniveus[6], Aspergillusflavus[15], Aspergillusphoenicis[4], Aspergillusfumigatus[15] and etc, and followed by yeasts such as Saccharomyces cerevisae[10] and bacteria such as Bacillus macerans[13]. Each type of microorganism requires a specific method to produce invertase enzyme and produced different level of enzyme production.According to Ashokumaret al. in year 2001[2], by using submerged fermentation of Aspergillusnigerstrain the maximum result is obtained as 18.3 U/ml for 120 hours fermentation time but the enzyme was optimized by undergo two step to yield 58.3 U/mi . In addition, this paper also had conducted an investigation on optimization condition of invertase production by using solid state fermentation. As a result, there is existed of invertase productivity which was 81.8 U/ml with less fermentation time that only required 72 hours yielding optimum productivity compares to submerged fermentation. However, this technique equire an additional step compare to submerged fermentation because the sample need to subjected under mechanical squeeze to obtain the extract before the sample undergo the centrifugation step for extracellular enzyme purpose. Therefore, this method becomes undesirable for this research due to the limitation in timeOther than the Ashokumaret al. research paper in year 2001[2] there are several research papers that used similar Aspergillusniger. Rubio and Navarro 2006 based on their own method the investigation of parameters such as pH, temperature and different carbon and nitrogen sources obtain a maximum enzyme activity in 30.84 U/mL.The maximum enzyme activity is obtained at 96 hours fermentation time at pH 3.5 and 30^0C using basal medium that contains 2% molasses as carbon sources supplemented by 0.5 % soya bean meal.According to Rubio and Navarro [12]investigation the effect of raffinose, Sucrose and turanose as carbon sources that obtain the maximum 4.0 U/mL enzyme productivity after 48 hours fermentation time. These indicate that the fermentation times are different under different cultivating conditions that influence the invertase production.

Besides the above strain, Aspergillusniveusis used to produce extracellular invertase by Guimaraeset al. in year 2009 [6] under submerged fermentation. This research paper is conducted to investigate the effect of agroindustrial residues as carbon sources for invertase production. Based on this paper, it is discussed that the presence of Sucrose in sugarcane bagasse contribute to the higher enzyme productivity compare to the presence of glucose which is stated that intracellular enzyme is the type of product that become greater compare to extracellular. Aspergillusflavusis one of the Aspergillusstrains which able to produce extracellular invertase. According to the Uma et al. in year 2010, this strain able to produce invertase and require four days to achieve optimum fermentation cultivating conditions at pH 5.0 and optimum temperature is 30^0C by using 3% inoculum size in CzapekDox using fruit peel waste as fermentation substrate. This species is culture by using submerged fermentation method that enhance by the addition of Sucrose and yeast extract for optimization purpose. Since this research use to purify the invertase therefore the optimum enzyme result is obtained in recovery process as 3.2 % and 5.8 fold.

Further review on the microorganisms used to produce this enzyme introduces another Aspergillusspecies strain that able to produce this enzyme. It is Aspergillusphoenicisthat use as a microorganism in Rustiguel[4]fermentation during year 2010. According to this research paper, this type of fungus was grown in Khanna medium that supplemented by Wheatbran as carbon sources at temperature 40^0C for 72 hours to obtain the optimum fermentation result. This research paper is similar to the previous reviews which used to purify the extracellular enzyme product to yield 12.5 fold enzymes with 72% recovery. However, the optimum condition that reported is at temperature 60^0C and pH 4.5.Aspergillusochraceusis another strain that able to produce this enzyme, according to Guimaraeset al. in year 2007[6], this fungus is able to produce extracellular invertase at maximum yield for 2.68 fold after the purification process. This optimum result is obtained after 96 hours fermentation period at temperature 40^0C by using Khanna medium. In contrast, the Bacillus maceransstrain is also able to be used as a fermentation microorganism to produce this enzyme but it is undergo a more complicated fermentation process. It is used by Samia in year 2008[13] using repeated batch fermentation method. This research had used immobilized Bacillus maceranscells in calcium alginate and used for the production of invertase. The purpose of her research is to investigate the influence of alginate concentration, cation concentration, cell to alginate ratio, initial cell loading, curing time and bead diameter on conversion of Sucrose to inverted syrup on fermentation product. She had used the immobilized cells in shake flasks study to consider the optimum parameter of her investigation. Finally, this researcher had proposed that the optimum parameter gained from the study to be 3% (w/v) sodium alginate, 3% (w/v) calcium chloride with 2 hours curing time, 200 alginate beads per flask with 2 mm bead diameter. This optimum parameter is based on fermentation of

immobilized cells of *Bacillus macerans*in alginate beads that is suggested as more efficient for the production of invertase and can be reused for seven cycles (336 hours) without any loss in their activity and 12 cycles with 72% residual activity.

From the above research papers this present study showed the better production of Invertase enzyme Utilising low cost agro waste materials. Although the commercial Sucrose gave good enzyme concentration Wheat bran and Ground nut cake flakes showedsignificantly moreenzyme activity hence these two suggested as a good substrate for future industrial production purposes.

Table 1: Qualytative Analysis Of Carbohydrate

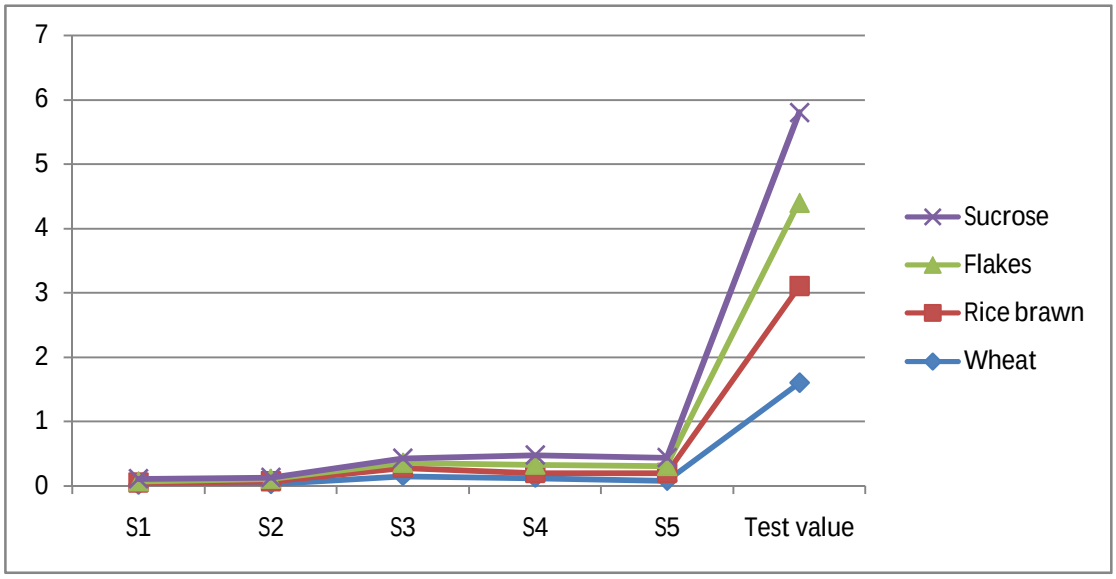
compounds	Wheat bran	Rice bran	Ground nut cake flakes flakes	Sucrose
Barfoed's test	+	+	+	+
Benedict's test for reducing sugar	+	+	+	+

TABLE 2: Enzyme Assay

Test sample	O.D value at 530 nm	Crude invertase U/ml
Wheat bran	0.23±0.1	72.4 U/ml
Rice brawn	0.39±0.2	43.5 U/ml
Ground nut cake flakes flakes	0.13±0.02	63.3U/ml
Sucrose	0.17±0.01	83.5U/ml

The OD Values are ± mean values.

Figure 1.protein analysis (lowry's method)



REFRENCES

[1] Alegre, A.C.P.,Polizeli, M.L.M. Jorge, H.F..J. and Guimaraes,L.H.S., (2009). Production of thermo stable invertase by Aspergilluscaespitosus under submerged or solid state fermentation using agro industrial residues as carbon source. Brazilian Journal of Microbiology. 40: 612-622.

[2] Ashokkumar, B., N.Kayalvizhi and Gunasekaran, P.2001. Optimization of media for â-fructofuranosidase production by Aspergillusniger in submerged and solid state fermentation Process Biochem. 37: 331-338.

[3] Benito, J.J., and Marshall, D.R.1981.Isozyme variation between species and form aespciales of the genus Puccinia.Can. J. Bot. 59: 2628-263

[4] ynthia Barbosa Rustiguel, Arthur Henrique avalcanti de Oliveira, Héctor Francisco Terenzi, JoãoAtílio Jorge , Luis Henrique Souza Guimarães. 2011.Biochemical properties of an extracellular β-D-fructofuranosidase II produced by Aspergillusphoenicis under Solid-Sate Fermentation using soy bran as substrate, Electronic Journal of Biotechnology Volume (14)

[5] New specifications prepared at the 57th JECFA (2001) and published in FNP 52 Add 9 (2001); previously prepared at the 15th JECFA (1971) as part of the specifications for "Carbohydrase from Saccharomyces species", published in FNP 52. The use of this enzyme was considered to be acceptable by the 57thJECFA (2001) if limited by Good Manufacturing Practice

[6] Guimaraes, L.H.S., A.F. Somera, H.F.Terenzi, M.L. Polizeli and Jorge J.A.2009.Production of âfructofuranosidasebyaspergillusniveususingagroindustrial residues as carbonsources: Characterization of anintracellular enzyme accumulated in thepresence of glucose. Pro. Biochem. 44:237-241

[7] Hasegawa, T.C., T. Hashimoto, J. Adachi,N. Iwabe and Miyata, T. 1993. Earlybranchings in the evolution ofeukarotes: ancient divergence ofEntamoeba that lacks mitochondriarevealed by protein sequence data. Mol. Evol. 36: 380-388.

[8] Kaur N and Sharma A D, 2005 Production, optimization and characterization of extracellular invertase by an actinomycete strain. Journal of Scientific and Ind.Research. Vol(64)

[9] Lowry O H,Rosenbrough N J,Fass A L,Randall R J,Protein measurement with Folin Phenol reagent.JBiolChem 193(1951):265-275

[10] Mahmoud Z. Sitohy , Mona M. Rashad ,Samy F. Sharobeem ,Abeer E. Mahmoud , Mohamed U. Nooman and Amr S. Al Kashe(2010) .Bioconversion of soy processing waste for production of surfactants.African Journal of Microbiology Research. Vol.4(24):pp. 2811-2821.

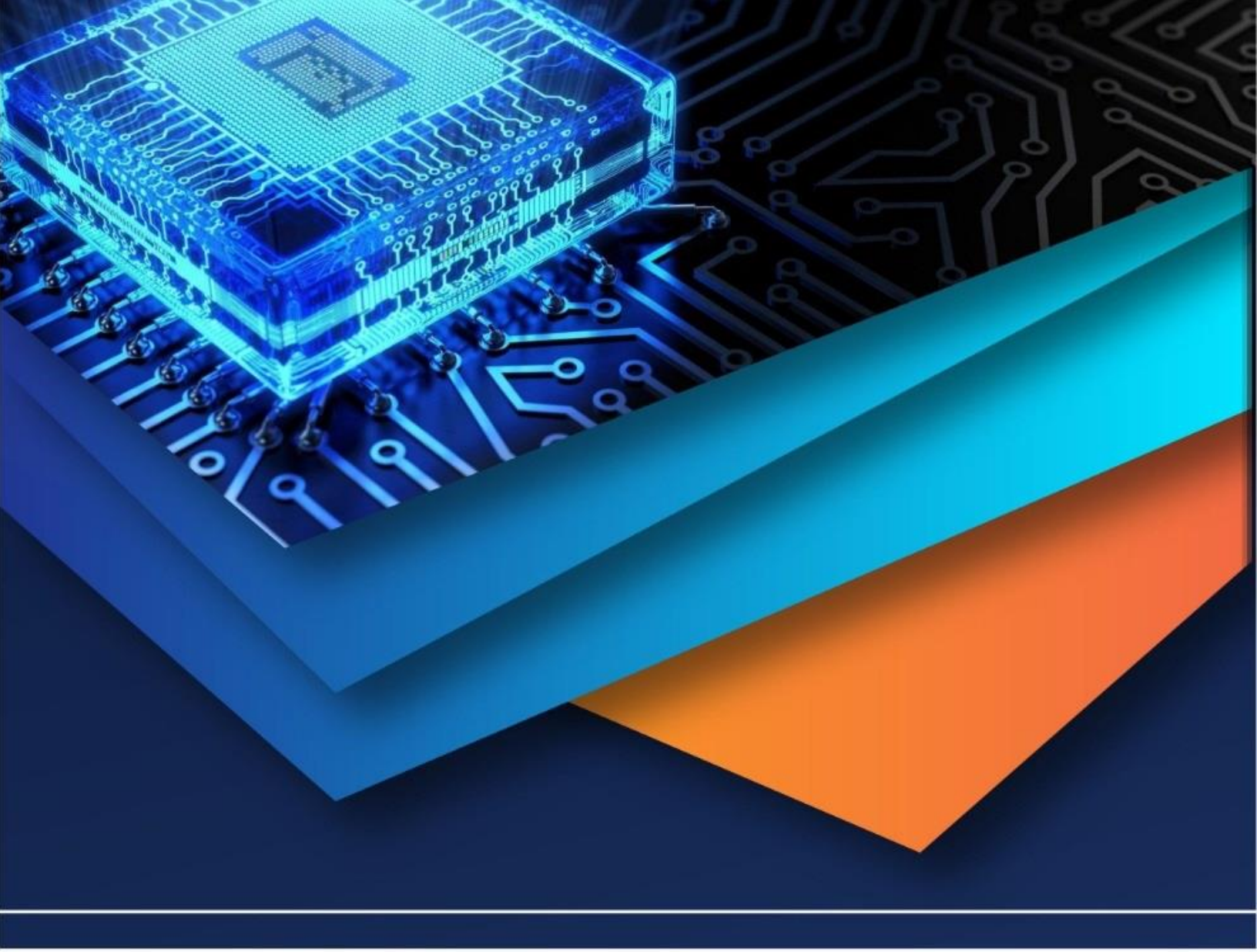
[11] Marquez L D S,Cabral B V,Feritas F F,Cardos V L,Ribeiro E J(2008).Journal of Molecular Catalysis B: Enzymatics.Volume (51):86-92

[12] Rubio, M.C., R. Runco and Navarro, A.R.2002. Invertase from a strain ofRhodotorulaglutinis. Phytochemistry.61: 605-609.

[13] Samia A. Ahmed, (2008). Invertase Production by Bacillus maceransImmobilized on Calcium Alginate Beads. Journal of Applied Sciences Research, 4(12): 1777-1781

[14] Silveira, M.C., E.M. Oliveira, E. Carvajaland Bon, E.P. 2000. Nitrogenregulation of Saccharomyces cerevisiaeinvertase. Role of the URE2 gene.Appl. Biochem. Biotechnol. 84- 86:247-254.

[15] Uma, C., D. Gomathi, D. Muthulakshmi and Gopalakrishnan, V.K. 2010.Production, Purification andCharacterization of InvertasebyAspergillusflavus Using Fruit PeelWaste as Substrate. Advan. Biolo. Res.4 (1): 31-36.



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