International Research Journal of Engineering and Technology (IRJET) e-ISSN: 2395-0056
Volume: 08 Issue: 06 | June 2021 www.irjet.net p-ISSN: 2395-0072

Hydrolysis of Starch Using Enzyme Produced Via Fermentation

Sourabh Pujari[1]

Student, Dept. of Biotechnology Engineering, KIT's College of Engineering, Kolhapur, India

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Abstract - *In this project we tried to find the best method for starch hydrolysis out of waste, i.e., from potato peels that can be an easily available waste source from chips manufacturing companies. We not only isolated the microbes producing starch degrading enzymes from potato peels but also used the peels as starch source for microbes' fermentation. As the concentration of enzymes thus obtained was very diluted due to other components contributing the broth composition, therefore we preferred using immobilization techniques: CLEAs and entrapment, i.e., using Na- alginate beads. In the end, comparative studies were done on each case, and the best alternative among the above is suggested.*

Key Words: **Enzyme, Amylase, Immobilization, CLEA's, Hydrolysis**

1. INTRODUCTION

Starch hydrolysis has numerous applications, a few being: differentiation of species of genera Corynebacterium, Clostridium, Bacillus, Bacteroides, Fusobacterium and members of Enterococcus spp.; in fermentation or food industry, for example, production of syrups, having a wide variation in composition of glucose, maltose and higher oligosaccharides, by means of enzymatic or acid hydrolysis of starch; etc. But chemical hydrolysis of starch involves chemicals which are hazardous for their exposure to nature. Therefore, alternative of biological hydrolysis has been found. In this project, starch hydrolysis using enzymes, obtained via fermentation, has been suggested. Potato, being a naturally occurring starch rich source, has been suspected to be surrounded by amylase- producing bacteria on its peels. So, isolation of amylase- producing bacteria from potato peels would lead to obtain significant amount of amylase out of waste. Moreover, for efficient utilization of enzymes, immobilization using CLEAs is found to be a better option compared to other immobilization techniques. This is so because, enzymes cross linking agents contribute to very less weight that in overall CLEA its weight is negligible, giving us almost 100 wt% enzyme proteins unlike in other immobilization techniques which lead to loss in enzyme recovery.

2. METHODOLOGY

2.1 Isolation of organism

Starch agar is a selective medium for the isolation of amylase producing bacteria. About 10 g of chopped potato is suspended in 10mL water overnight. The sample is then serially diluted in sterile physiological saline and dilution was done up to 10-5 by thorough mixing. 0.1ml of sample from 10-3 dilution is spread on sterile petri dishes containing starch agar with the help of L-rod and the plates are incubated at 37°C for 24-48 hours. After incubation the plates are observed for the growth of bacteria.

Fig -1: Streak plate of the isolated bacteria

2.2 Determination of amylase activity

All the microbial isolates are tested for amylase production by starch agar. Starch agar medium inoculated with the organism is subsequently flooded with iodine solution. After 72 hrs of growth the starch plate are flooded with the above iodine solution. Cleared zone are seen around amylase producing colonies under blue background.

2.3 Inoculation

Suspension of high clearance giving zone in starch broth is done. It is incubated in 20 mL starch broth at 280C and then transferred to fermentation flask after 24 hrs. Biomass formation is observed after 72 hours of incubation. The suspensions are prepared and the pellet (60 gm) of microbes is collected in clean beakers after the centrifugation.

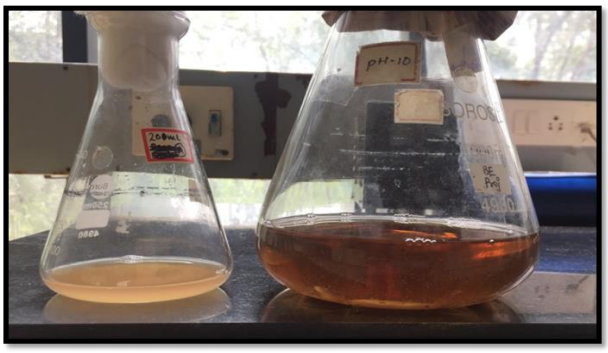

Fig -2: Seed culture and the production flask.

2.4 Removal of solids from broth

Culture broth was centrifuged as 4500 for 10mins. The supernatant was separated and was assayed for -Amylase activity in each case.

2.5 Immobilization using CLEAs

Saturated ammonium sulfate solution was added up to the final concentration of 70% saturation in 2 ml crude enzyme solution to precipitate the enzyme. After 30 min, glutaraldehyde was added slowly to the final concentration of 2 %(v/v) to cross link enzyme precipitate. The mixture was kept at 20°C for 6h cross linking reaction with continuous stirring at 150 rpm. Subsequently, the mixture was centrifuged at 13,000g for 15 min at 4°C. The recovered pellet was washed three times with 0.1M sodium phosphate buffer, pH 7 to remove unreacted glutaraldehyde and suspended in buffer. Activity recovery in CLEAs was calculated as given in following equation. Activity recovery (%) = (Total activity of CLEA in units/Total crude enzyme activity used for CLEA Preparation in units) × 100

Fig -3: The CLEAs have settled at the bottom of the centrifuge tube

2.6 Immobilization using Sodium Alginate Beads

Preparation of sodium alginate: 0.25M CaCl2 preparation: Sodium alginate solution was mixed with breakers separately. 2ml of the above solution was pipette out by using sterile syringes. The solution was injected into the beaker containing CaCl2 solution as drop wise. The beads were prepared and stored in a refrigerator

2.7 Enzyme assay

Amylase activity were assayed by measuring the amount of reducing sugars released from starch using dinitro salicylic acid (DNSA) method (Bernfeld 1995).

2.7.1 Estimation of glucose by dinitrosalicylic acid (DNSA)

Dinitro salicylic acid method is a simple and sensitive method.

2.7.2 Procedure

Pipette out 0.0 ml (blank) to 3.0 ml of sugar solution into clean test tube. Make up the final volume to 3.0 ml with d.H2O in all the tubes. To each tube add 3.0 ml of dinitro salicylic acid reagent and cover the tube with aluminum foil. Place the tube in boiling water for 6 minutes and cool them in a containing tap water. Read the intensity at 575 nm (green filter). Plot a graph for series of the standard glucose solution

2.7.3 Calculation

Calculate the amount of reducing sugar present in the sample using standard graph.

2.7.4 Estimation of glucose

The amount of glucose present per ml in o - amylase assay mixture was calculated from the standard graph.

$$\text{Amount of glucose} = \frac{\text{OD of standard solution} \times \text{Concentration of standard solution}}{\text{OD of unknown solution}}$$

The amount of glucose was expressed as µ gm / ml.

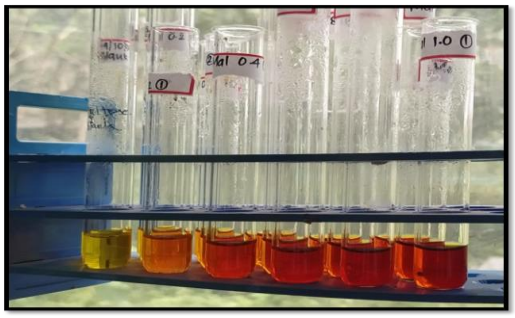

Fig -4: Pictures of maltose Standard curve test and the free enzyme assay

2.8 Calculation of enzyme activity

The Enzyme activity was calculates as
Concentration of the product produced/molecular weight of the product = 1/ incubation period the enzyme activity is expressed as µ moles / ml/ min.

3. RESULT AND ANALYSIS

3.1 Maltose Standard Graph

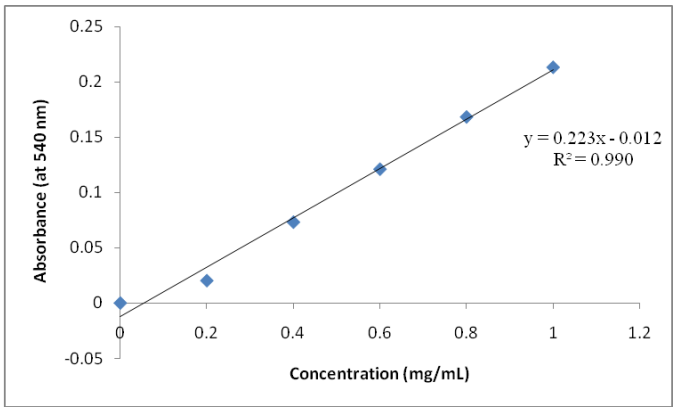

Chart-1: Maltose Standard Graph

Table -1: Concentration vs. respective O. D

Sr. No.	Maltose Conc. (mg/mL)	Absorbance (at 540 nm)	
1.	0	0	
2.	0.2	0.0203	
3.	0.4	0.0733	
4.	0.6	0.1213	
5.	0.8	0.1686	
6.	1	0.2136	
Pure enzyme	Un Pe	0.165	
Enzyme in broth 1	Un Br	0.188	0.184 (avg. of 1, 2, 3)
Enzyme in broth 2		0.132	
Enzyme in broth 3		0.198	
Na- alginate immobilized enzyme	Un Na	0.026	
CLEAs	Un Cl	0.1726	

3.2. Calculations

Activity of free enzymes in broth
From graph: y = 0.223x - 0.012
Here, y (O. D.) = 0.184
→ x (Un Br) = 0.8789 mg = 2.5698 µmol (maltose)

$$\text{Therefore, Activity} = \frac{mol\ of\ product\ formed}{tot.vol \times incubation\ time} = \frac{2.5698\ \mu mol}{2\ mL \times 10\ min}$$

= 0.1285 µmol/mL min

Activity of enzymes immobilized in alginate beads
From graph: y = 0.223x - 0.012
Here, y (O. D.) = 0.026
→ x (Un Na) = 0.170 mg = 0.4970 µmol (maltose)

$$\text{Therefore, Activity} = \frac{mol\ of\ product\ formed}{tot.vol \times incubation\ time} = \frac{0.4970\ \mu mol}{2\ mL \times 10\ min}$$

= 0.0248 µmol/mL min

Activity of CLEAs
From graph: y = 0.59 x + 0.005
Here, y (O. D.) = 0.1726
→ x (Un Cl) = 0.2840 mg = 0.8306 µmol (maltose)

$$\text{Therefore, Activity} = \frac{mol\ of\ product\ formed}{tot.vol \times incubation\ time} = \frac{0.8306\ \mu mol}{2\ mL \times 10\ min}$$

= 0.0415 µmol/mL min

Hence, the enzyme activity obtained from free enzymes, enzymes immobilized in alginate beads and CLEAs were 0.1285 µmol/mL min, 0.0248 µmol/mL min and 0.0415 µmol/mL min respectively.

4. CONCLUSION

Enzyme was prepared by fermentation pathway using bacteria isolated from potato peels which is usually an industrial waste which makes this method eco-friendly and economical at the same time. The optimum performance of the enzyme was found to be in the form of CLEAs because they showed high enzyme activity as well as reusability.

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