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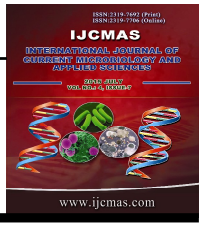

Original Research Article

Extraction of Enzymes from Potato Peels Substrate using *Bacillus subtilis*

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Keywords

Potato Peels, *Bacillus subtilis*, Extraction of Enzymes

A B S T R A C T

Here the work contains Extraction of Enzymes from Potato Peels Substrate Using Bacillus Subtilis. The work was done to study the growth habit of microbes on standard media, standardize the conditions for growth of microbes using potato peel & and measure the efficacy of filtrate as enzyme source. Experimental details were like Design-CRD, 3 replication & 8 treatments. Bacterial strains were used like B_1 = *B. subtilis*, B_2= No bacteria, Incubation temperature T_1 = 37^0C, T_2 = Room Temperature (R.T.) and Incubation Hours H_1 = 24, H_2 = 48. Bacterial strain Bacillus subtilis consume maximum amount of starch (96.27 mg/g) as compare to other treatments. Amylase and protease enzymes activity also found in highest in treatment. All the results were within accepted criteria.

Introduction

Potato *(Solanum tuberosum L.)* is an important crop in India. It is a primary source of starch for vegetarian diet. In India, it was cultivated in an area of about 1.83 million hectares with production of 34.39 Million Tonne in 2008-09, whereas in Gujarat in the same year it was cultivated in 57,000 hectares with production of 4.21 Million Tonne (www. agricoop.gov.in). There are about 35 potato processing units in Gujarat foods, Rajkot is one of the biggest units (www.potatopro.com). This unit was visited by the staff members of scheme, to get information about the utilization of potato peel – a waste product of wafer making unit. The capacity of the plant is about 150 tonnes potato per day, and potato peels production is about 30 tonnes potato peel @ 200 g / kg potato. Presently the potato peels are mainly used as a composting material in farm as well as selling for the cattle feed (according to Balaji foods (P.) Ltd, Rajkot).

Most *Bacillus* and some other species of bacteria have a wide range of hydrolytic enzyme systems and are often capable of utilizing the organic matter consisting of complex mixtures; moreover, with the exception of the certain group of bacteria, they are harmless microbes which are included in the groups of organisms generally recognized as saprophytes. These organisms are easy to grow and require no expensive growth factors. They are able to produce extra cellular enzymes in high concentrations which can isolate. The *Bacillus* will also be

tried for the extraction of extra cellular enzymes from potato peels. The extra cellular enzymes are used for a variety of different purposes, including starch modification in the paper & pulp industries, brewing, pharmaceutical industries, leather production industries etc. The wastes coming out from potato plants in the form of liquid and solid arise from peeling, trimming, slicing, cleaning, and rinsing operations which creates pollution problem.

Looking to the above it was thought to utilize the byproduct such as potato peels of the potato industries for the extraction of extra cellular enzyme and also to solve the problem of pollution. There are some review are done for same case (Nurullah and Fikret, 2011; Ashlee *et al.,* 2008; Reda *et al.,* 2008; Ashwini *et al.,* 2011; Bushra *et al.,* 2007Chun-lei Wang *et al.,* 2010; Paula and Pérola, 2010; Archana *et al.,* 2010; Muhammad *et al.,* 2011; Korsten and Cook, 1996; Mahmood *et al.,* 1998; Carlos and Meire, 2009; Sasmita and Niranjan, 2008; Mona and Amani, 2008; Onilude *et al.,* 2012; Saraswati *et al.,* 2012; Tamires *et al.,* 2012; Viggo *et al.,* 1995).

Materials and Methods

For obtaining correct situation about potato peel as waste materials, AICRP on PHT staff made an industrial visit at Apricot foods industries, Rajkot and Rajen wafers, Rajkot. Huge amount of peels were produced and the main consumers of that was farmers used for the cattle feed and also used for bio-fertilizers.

Bacterial cultures obtained from Microbial Type Culture Collection Centre (MTCC), Chandigarh. MTCC is a culture collection bank i.e. different cultures having different usefulness. Potato peel act as source of starch obtained from local market of Junagadh i.e. from Sagar Namkeen. For standardize the conditions for growth of microbes it is necessary to maintain Potato peel and nutrient broth (a chemical) ratio and a continual practice it was maintained at 1:10. For 1 gram of potato peel substrate it is necessary to take 10 ml of nutrient broth.

Process

Make growth of bacterial cultures on Nutrient agar media & after it on Peel nutrient agar media then Starch hydrolyzation was confirmed by iodine test and after the confirmation, the both bacterial cultures are inoculated in a sterile nutrient broths separately. After 24 hrs incubation, 0.1 ml bacterial cultures from nutrient broth inoculated in the treatments T1 to T8 (*Bacillus subtilis* culture added in T1 to T4 flasks) while T5 to T9 treatment flasks were without inoculated (T5 to T8 flasks act as control treatments) and put all the flasks at it for their respective temperature and time.

After that the flasks material was centrifuged at 2000 rpm for 10 minutes for obtaining crude enzymes. Enzyme assay of supernatant was made and the absorbance was measured (Optical Density) at their respective wavelength. Starch consumption data are also carried out. Microbiological growth media used for the growth of bacteria like Nutrient agar media, starch agar media, peel agar media and peel nutrient broth.

Results and Discussion

Growth habit of bacteria (*B. subtilis*) on nutrient agar media, starch agar media & on peel agar media

Standardization of the conditions for growth of bacteria using potato peel

As per the above results it is concluded that

the pH of peel agar media was maintained at 7.9 and the ratio of potato peel substrate + nutrient agar / broth was maintained 1:10. i.e. 10 gram of potato peel substrate was necessary for 100 ml of nutrient agar / broth for better hydrolysis of starch by *Bacillus* type of bacteria.

Measurement of the enzyme efficacy

A general formula for glucose or enzyme production was carried out as follow:

$$\text{Glucose produced} = \frac{\text{Graph factor x Sample O.D. x raw sample (in g) x Dilution factor}}{\text{Aliquote taken x 1000}}$$

Standard glucose estimation by Anthrone method

100 mg in 100 ml distilled water. Working standard - 10 ml of stock diluted to 100 ml with distilled water and estimation is carried out by Anthrone method. Graph factor obtained by this estimation is very useful for starch consumption.

Table 6 revealed that starch consumption was found higher (96.27 mg/g) in treatment T4 i.e. bacterial strain *B. subtilis* consume maximum amount of starch at room temperature during 48 hours of time period.

Table.1 Proximate composition of potato peel*

Component (%)	Potato waste (peel)
Starch	66.78
Dry matter	17.82
Alcohol-insoluble solids	62.70
Total soluble sugars	1.40
Reducing sugars	0.91
Cellulose	2.20
Crude protein	14.70
pH	5.99

* The data obtained from United States department of Agricultural national nutrient database (USDA 2009)

Table.2 Treatment explanation

Sr. No.	Treatments	Bacteria Strains	Incubation Temperature	Incubation Hours
T1	$B_1T_1H_1$	*B. subtilis*	37^0C	24
T2	$B_1T_2H_1$	*B. subtilis*	R.T.	24
T3	$B_1T_1H_2$	*B. subtilis*	37^0C	48
T4	$B_1T_2H_2$	*B. subtilis*	R.T.	48
T5 (control)	$B_0T_1H_1$	No bacteria	37^0C	24
T6(control)	$B_0T_2H_1$	No bacteria	R.T.	24
T7 (control)	$B_0T_1H_2$	No bacteria	37^0C	48
T8 (control)	$B_0T_2H_2$	No bacteria	R.T.	48

Table.3 Colony morphology & Microscopic analysis of *Bacillus* group of bacteria on N-agar media

Colony	**Colony characteristics on Nutrient agar media**				**Bacterial strain**	**Microscopic analysis**
	Form	**Elevation**	**Colour**	**Margin**		
1	Circular	Umbonate	Creamy off white	Undulate	*Bacillus subtilis*	Gram positive rods

Standardize the conditions for growth of bacteria using potato peel

Table.4 Growth of bacteria on peel agar media

Sr. no.	pH	Potato peel (in gram)	Average growth of ***Bacillus subtilis*** (in CFU/ml)
1	7.5	5	1.07×10^7
2	7.5	10	1.83×10^7
3	7.5	20	- peel ratio is high, so agar can't properly solidify and bacterial growth can't observe properly -
4	7.7	5	1.28×10^7
5	7.7	10	2.09×10^7
6	7.7	20	- peel ratio is high, so agar can't properly solidify and bacterial growth can't observe properly -
7	7.9	5	1.66×10^7
8	7.9	10	2.56×10^7
9	7.9	20	- peel ratio is high, so agar can't properly solidify and bacterial growth can't observe properly -

Table.5 Optical Density and graf factor of standard glucose determined by Anthrone method

ml	concentration in µg	O.D.	O.D./ml	graf factor
0.00	0.00	0.000	0.00	129.87
0.2	20	0.150	0.750	
0.4	40	0.357	0.893	
0.6	60	0.461	0.769	
0.8	80	0.698	0.873	
1	100	0.749	0.749	

Table.6 Starch consumption by bacteria in various treatments

Sr. No.	Treatments	Average Glucose produced (in mg/g)	Starch consumed = glucose produced x 0.9 (in mg/g)
T1	$B_1T_1H_1$	62.58	56.32
T2	$B_1T_2H_1$	87.4	78.66
T3	$B_1T_1H_2$	82.27	74.05
T4	$B_1T_2H_2$	106.97	96.27
T5 (control)	$B_0T_1H_1$	0.00	0.00
T6 (control)	$B_0T_2H_1$	0.00	0.00
T7 (control)	$B_0T_1H_2$	0.00	0.00
T8 (control)	$B_0T_2H_2$	0.00	0.00

Table.7 Optical Density and graf factor of standard glucose determined by DNSA method

Ml	concentration in µg	O.D.	O.D./ml	graf factor
0.00	0.00	0.000	0.00	158.5624
0.2	20	0.136	0.682	
0.4	40	0.265	0.663	
0.5	50	0.338	0.676	
0.6	60	0.473	0.788	
0.8	80	0.550	0.688	
1	100	0.625	0.625	

Table.8 Amylase production by bacteria in various treatments

Sr. No.	Treatments	Average Amylase enzyme produced (in mg/g)
T1	$B_1T_1H_1$	23.92
T2	$B_1T_2H_1$	37.24
T3	$B_1T_1H_2$	35.65
T4	$B_1T_2H_2$	56.9
T5 (control)	$B_0T_1H_1$	0.00
T6 (control)	$B_0T_2H_1$	0.00
T7 (control)	$B_0T_1H_2$	0.00
T8 (control)	$B_0T_2H_2$	0.00

Table.9 Protease production by bacteria in various treatments

Sr. No.	Treatments	Average O.D. at 440 nm	Treatment average – control average	Protease enzyme produced = (Treatment average – control average) x 0.2 (in mg/g)
T1	$B_1T_1H_1$	0.381	0.309 (T1-T9)	0.06
T2	$B_1T_2H_1$	0.415	0.371 (T2-T10)	0.07
T3	$B_1T_1H_2$	0.545	0.481 (T3-T11)	0.10
T4	$B_1T_2H_2$	0.938	0.893 (T4-T12)	0.18
T5 (control)	$B_0T_1H_1$	0.072	-	0.00
T6 (control)	$B_0T_2H_1$	0.044	-	0.00
T7 (control)	$B_0T_1H_2$	0.064	-	0.00
T8 (control)	$B_0T_2H_2$	0.045	-	0.00

Table.10 Statistical analysis of individual factors as well as interaction

Effect	**Starch Consumption**	**Amylase production**	**Protease production**
Bacterial strain (B)			
Bacillus subtilis (B_1)	76.325	40.491	0.513
No bacteria (B_0)	0.000	0.000	0.000
Incubation temperature (T)			
37 ^{o}C (T_1)	37.136	14.045	0.164
Room Temperature (T_2)	49.335	22.225	0.344
Incubation hours (H)			
24 (H_1)	34.816	13.854	0.193
48 (H_2)	51.656	22.416	0.315

Table.11 Brief results regarding various treatments

Sr. No.	Treatments	Starch consumption (in mg/g)	Amylase enzyme activity (in mg/g)	Protease enzyme activity (in mg/g)
T1	$B_1T_1H_1$	56.32	23.92	0.06
T2	$B_1T_2H_1$	78.66	37.24	0.07
T3	$B_1T_1H_2$	74.05	35.65	0.10
T4	$B_1T_2H_2$	96.27	56.9	0.18
T5 (control)	$B_0T_1H_1$	0.00	0.00	0.00
T6 (control)	$B_0T_2H_1$	0.00	0.00	0.00
T7 (control)	$B_0T_1H_2$	0.00	0.00	0.00
T8 (control)	$B_0T_2H_2$	0.00	0.00	0.00

Fig.1 Graph of Concentration v/s O.D. of standard glucose determined by Anthrone method

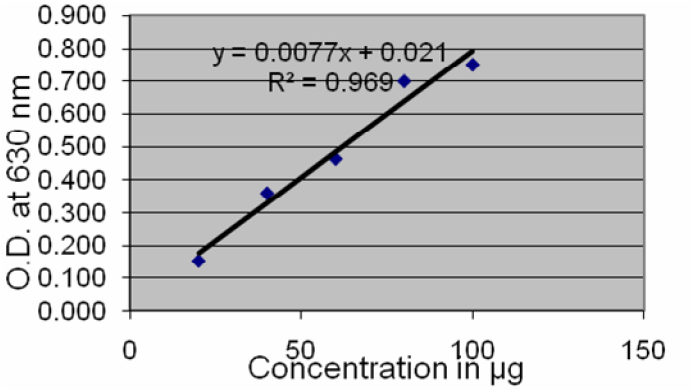
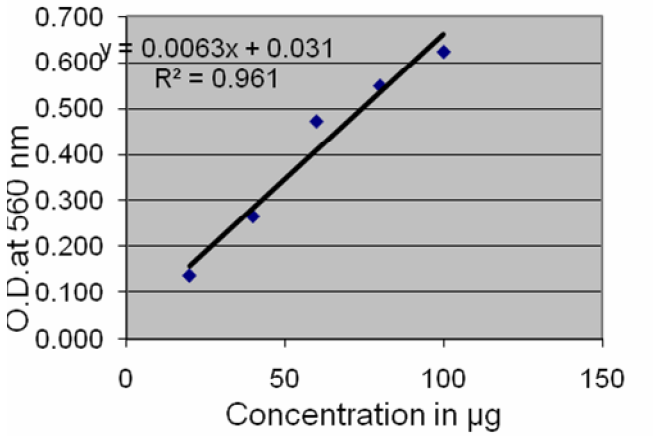


Fig.2 Graph of Concentration v/s O.D. of standard glucose determined by DNSA method



Standard glucose estimation by DNSA method

100 mg glucose dissolved in 100 ml distilled water. Working standard - 10 ml of stock diluted to 100 ml with distilled water and estimation was carried out by Di Nitro Salicylicylic Acid (DNSA) method. Graph factor obtained by this estimation is very useful for estimation of amylases enzyme activity.

Table 8 reveals that α - Amylase production was found higher, 56.9 mg/g, in treatment T4 i.e. bacterial strain *B. subtilis* produce more amount of α - amylase enzyme at room temperature during 48 hours of time period.

Table 9 reveals that Protease production was found higher, 0.18 mg/g, in treatment T4, i.e. bacterial strain *B. subtilis* produce more amount of protease enzyme at room temperature during 48 hours of time period.

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