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EXTRACTION AND OPTIMIZATION OF LIPASE USING *Aspergillus niger* BY SOLID STATE FERMENTATION

VIJAYANAND S*, USHA AND RAGHAVI

Department of Life Sciences, Kristu Jayanti College (Autonomous) Bengaluru

***Corresponding Author: Vijayanand S: E Mail: vijayanand78bio@rediffmail.com**

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ABSTRACT

Microbial lipase is majorly produced by Submerged Fermentation (SmF), which is well known for the engineering aspects that's currently being developed. However Solid State Fermentation (SSF) has shown some advantages over Submerged Fermentation in production of enzyme comparatively, even at the commercial scale. Solid state fermentation holds tremendous potential for the production of the enzyme Lipase by *Aspergillus niger.* Solid Substrate like Wheat bran, Gingelly oil cake which is rich in starch was used. These agro industrial residues are cheap raw materials for the production of lipase. *Aspergillus niger* was identified to be the best producer of lipase. When *Aspergillus niger* was incubated at room temperature for 6 days it showed a high yield of lipase in gingelly oil cake substrate in solid state fermentation. The enzyme showed remarkable stability in presence of detergents and it could be effectively used for removal of triglyceride oils in laundry.

Keywords: Solid state fermentation, Lipase, *Aspergillus niger*, Solid substrate (Wheat bran, Gingely oil cake), Detergents, Triglyceride oils

INTRODUCTION

Lipase is a triacylglycerol acyl hydrolase enzyme that catalyzes the hydrolysis and also synthesis of esters. Some lipase is capable of catalyzing the esterification, trans-esterification and interesterification of lipids. Lipase is the enzyme that is seen in plants,

animals and also in micro organisms. But only microbial lipase can be produced in large amount, by large scale production.

These are extensively applicable in several another industries such as dairy, food and beverage, animal feed, cleaning, biofuel, pharmaceuticals, textile cosmetic, perfumery, flavour industry, biocatalytic resolution, esters and amino acid derivatives, fine chemicals production, agrochemicals, biosensor, and bioremediation **[1-3]**. Microbial lipase is majorly produced by Submerged Fermentation (SmF), which is well known for the engineering aspects that's currently being developed. However Solid State Fermentation (SSF) has shown some advantages over Submerged Fermentation in production of enzyme comparatively, even at the commercial scale. Microbial lipases are produced mostly by submerged culture **[4]**, but solid state fermentation methods **[5]**. The concept of using this is it has shown much promise in development of several bioprocesses and products. Solid state offers greatest possibilities when fungi are used. Unlike other microorganisms, fungi typically grow in nature on solid substrates such as rice bran, wheat bran, gingelly oil cake, i.e. low in moisture. In SSF, the moisture necessary for microbial growth exists in an absorbed state or I complex with solid matrix. In SSF, the substrate itself acts as a carbon source and occurs in absence or near absence of free water. However, in solid state fermentation, the process occurs in absence or near absence of free water by employing a natural substrate or inert substrate or solid support. The aim of SSF is to bring cultivated fungi in tight contact with the insoluble substrate and to achieve the highest nutrient concentration from the substrate for fermentation.

Enzyme production is one of the most important applications of SSF. SSF has advantage over submerged fermentation such as high volumetric productivity, low cost of equipment involved, better yield product, lesser waste generation and lesser time-consuming processes etc.

In recent years, considerable increase in lipase production from microbes and agro industrial wastes using SSF has gained importance. However, fungi are the best lipase producers. Most used agro waste residues are wheat bran and gingelly oil cake for maximum lipase production by *Aspergillus niger*. Hence, the selection of an appropriate substrate is an essential step during the production of enzymes in SSF. Various agro-industrial residues such as wheat bran, soybean cake, rice husk, gingelly oil cake, olive oil cake, sugar cane bagasse,

babassu oil cake, and sheanut cake were studied **[6-8]**.

Among many micro organisms, filamentous fungi are considered to be most suitable for process involving solid substrate fermentation due to their ability to grow with a low amount of free water, together with their efficiency to degrade some pollutants. Especially, the production of lipolytic enzymes by filamentous fungi in solid substrate fermentation from agro industrial wastes had attracted with great interest, since these enzymes can be extracted easily from fermentation medium as they are mostly extracellular enzymes.

Fungi of genus *Rhizopus, Mucor, Rhizomucor, Geotichum, Penicillium* and *Aspergillus* are reported to be lipase producing organisms. Among which the *Aspergillus niger*, a fungus and one of the most common species of genus *Aspergillus* is considered to be prominent for the synthesis of lipase with industrial applications, because of its ability to grow rapidly on the solid support, and synthesis large amount of lipase. Therefore this study is aimed to evaluate the production of lipase by *Aspergillus niger* grown in solid substrate fermentation using agro industrial waste supplements such as wheat bran and gingelly oil cake. Also estimation of enzyme is done by following the procedure of Lowry's method and enzyme activity is calculated using titrimetric assay Procedure.

MATERIALS AND METHODS

Production of Inoculum

All the components from A to C were sterilized separately at 121 ˚C, 15 psi for 15 minutes. Production medium was prepared using the solution. The medium was then inoculated by adding spore suspension. The inoculated medium was incubated at 30 ˚C in an incubator for 24 hours. After 24 hours, a ribbon like mass had been formed in the medium i.e. mycelium. Then mycelium was taken out using forceps and suspended in sterile distilled water and homogenized. This was directly used as inoculums **(Image 1, 2)**.

Preparation of solid substrate:

Rice bran (7.5gm) and gingelly oil cake (2.5 gm) are taken in a clean sterilized conical flask and 15ml of distilled water was added. It was mixed well with a clean glass rod and plugged with cotton. Distilled water can also be replaced by 0.5%, 1%, 1.5% saline solution for fermentation process. And the contents were sterilized in 10ml down to process temperature. To the slants add 2 ml of sterile distilled water or the saline solution was added and spores were transferred to the flasks. The flasks were kept for incubation at 30 ˚C. Samples were

withdrawn at regular time intervals and the lipase activity was determined **[9, 10]**.

Extraction of enzyme:

At the end of the fermentation period 1 gm of solid /moldy substrate was homogenized with 10ml of distilled water using mortar and pestle. Then, the filtrate was centrifuged at 10,000 rpm for 20 minutes. The supernatant was used as enzyme source.

This was the conventional method of extraction, but instead of using water for extraction, we used 0.5% saline, 1% saline, 1.5% saline, 2% saline solution in different set of experiments.

Enzyme assay:

Lipase activity was determined by using the modified titrimetric method based on the titration with 0.05 N NaOH of the fatty acids released from the hydrolysis of olive oil, previously emulsified with xanthan gum, by the action of the lipase present in the fermentation extract **[11]**. Titrimetric method measures the rate of neutralization of Sodium hydroxide by released fatty acids as a function of time **Ota and Yamaoda, 1966** have reported a titrimetric method using olive oil as a substrate and polyvinyl as a substrate **(Image 3, 4)**.

Olive oil emulsion preparation: Sonicate the mixture of 5gm of olive oil and 5ml of 5% triton X-100 for 10 minutes. To the olive oil emulsion add 25ml of 4% BSA solution and 15ml of 0.1M Potassium phosphate buffer, at pH-7.0.

$$\text{Lipase activity} = \text{Vol. of NaOH} \times \frac{\text{normality of NaOH} \times 1000 \times 2}{\text{Time for incubation (units/ml/min)}}$$

Volume of NaOH=Test-Control

One unit of lipase activity: It is defined as one micromole of free fatty acid liberated per ml of enzyme per minute.

Protein Estimation: **(Image 5)**

$$\text{Specific activity} = \frac{\text{Total activity}}{\text{Total protein}}$$

Partial purification of crude lipase

Partial purification of the crude lipase can be done by several methods such as, ammonium sulphate precipitation, Dialysis method, aqueous two phase extraction

Ammonium sulphate precipitation:

The culture precipitate of *Aspergillus niger* was concentrated by precipitating with 40%, 50% and 70% ammonium sulphate at 4℃. The resultant mixture was centrifuged at 10,000rpm for 20 minutes. The pellet was resuspended on 0.5M phosphate buffer at ph-6.5. The enzyme activity and protein content were checked for the pellet and supernatant.

Partial Purification of Lipase by Column chromatography

Column chromatography was used for separation of solvents as per standard procedure. The column was packed with

hexane solution by slowly adding silica gel to the column. The column was packed in such a way that no air bubbles are formed. After the column is packed the solvent is added above the gel level. The sample was added to the column and the elution time and elution volume was noted. The rate flow is adjusted to 1 drop /2sec **(Image 6)**.

Image 1: Preparation of slants

Image 2: Mycelium mat formation

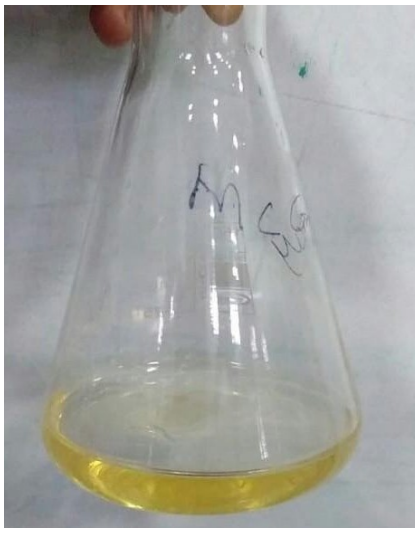

Image 3: Before titration

Image 4: End point of the titration

Image 5: Protein estimation

Image 6: Column chromatography

Image 7: Eluent and Eluate

RESULT AND DISCUSSION

Calculations:

$$\text{Lipase Activity} = \frac{\text{Vol. Of NaOH} \times \text{Normality of NaOH} \times 1000 \times 2}{\text{Time of Incubation}}$$

$$= \frac{0.3 \times 0.1 \times 1000 \times 2}{20}$$

$$= 3 \text{ units/ml/minute}$$

Hence, the obtained Lipase Activity was found to be 3 units/ml/minute.

Table 1: Enzyme Activity

SERIAL NO	INITIAL	FINIAL
CONTROL VALUE	0.0	0.1(ml)
TEST VALUE	INITIAL	FINIAL
SALINE CONC:0.5%	0.0	0.4(ml)
SALINE CONC: 1%	0.0	0.4(ml)
SALINE CONC: 1.5%	0.0	0.4(ml)
SALINE CONC:2%	0.0	0.4(ml)
SALINE CONC:2.5%	0.0	0.4(ML)

Test value- Control value = 0.4- 0.1= 0.3(ml)

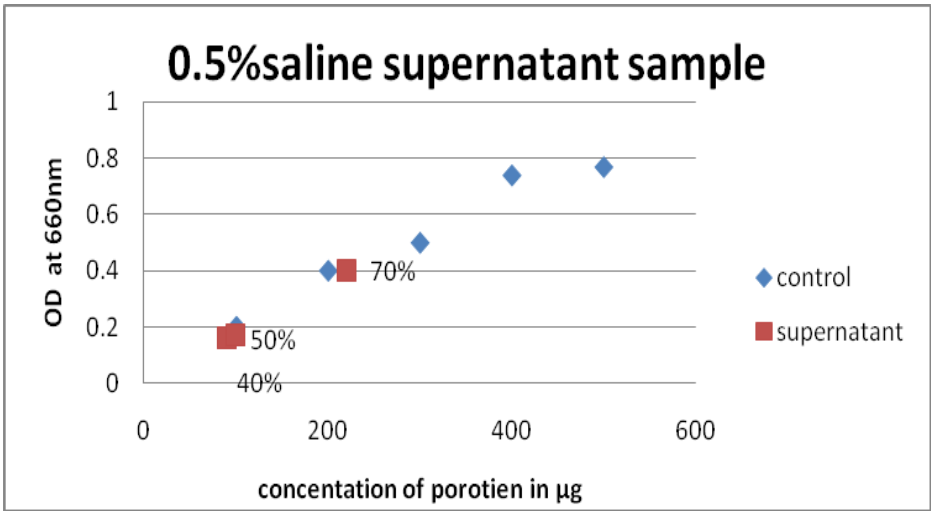

Figure No 1- 0.5% saline supernatant graph

Table 2

AMMONIUM SULPHATE CONCENTRATIO	VOL OF SUPERNATANT SAMPLE OF 0.5% SALINE CONC	OD VALUE AT 660NM
40%	0.2	0.16
50%	0.2	0.17
70%	0.2	0.40

For 40 %, 50 % and 70 % ammonium Sulphate the Specific Activity of enzyme was found to be 0.9, 0., 1 and 2.2 µmole/mg/min respectively

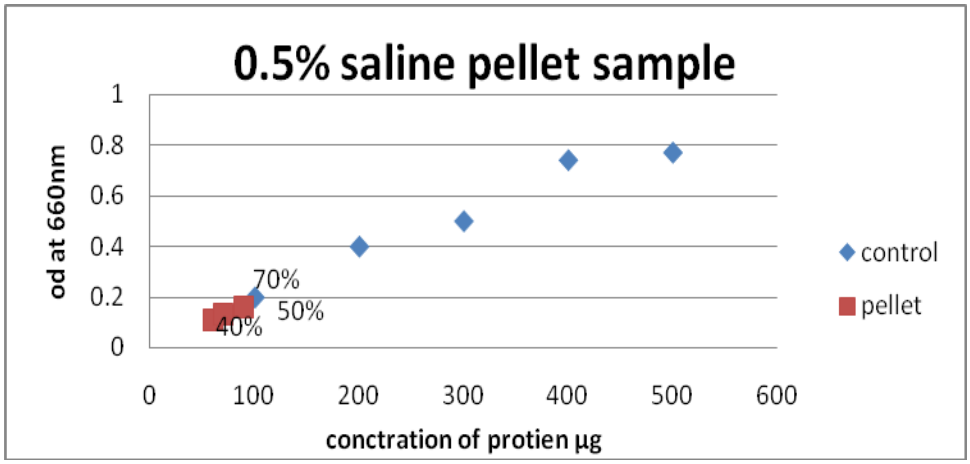

Figure 2: 0.5% saline pellet graph

Table 3

CONCENTRATION OF AMMONIUM SULPHATE	VOLUME OF PELLET SAMPLE 0F 0.5 % SALINE CONC(ML)	OD AT 660NM
40%	0.2	0.11
50%	0.2	0.13
70%	0.2	0.16

For 40 %, 50 % and 70 % ammonium Sulphate the Specific Activity of enzyme was found to be 6, 7.5 and 9µmole/mg/min respectively

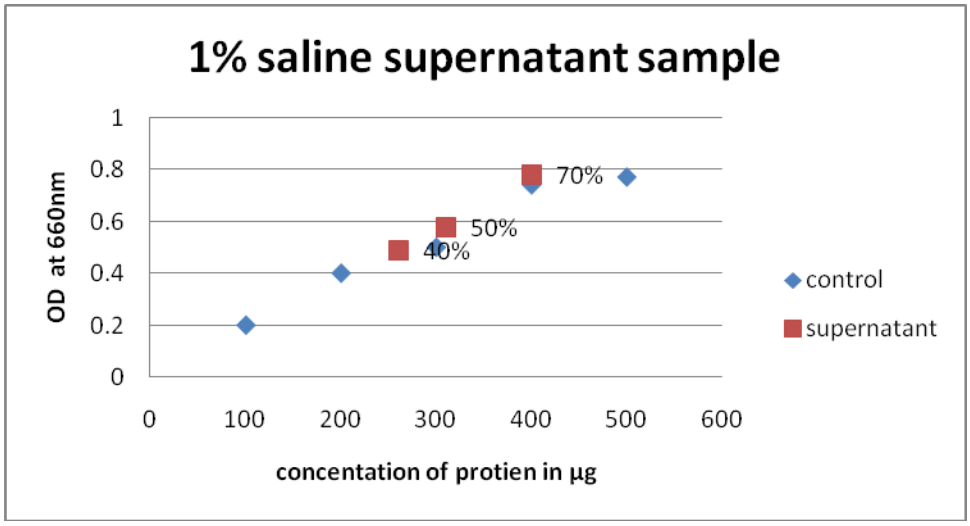

Figure 3: 1% saline supernatant graph

Table 4

CONCENTRATION OF AMMONIUM SULPHATE	VOLUME OFSUPERNATANT OF 1% SALINE CONC (ML)	OD AT 660NM
40%	0.2	O.49
50%	0.2	0.58
70%	0.2	0.75

For 40 %, 50 % and 70 % ammonium Sulphate the Specific Activity of enzyme was found to be 2.6, 3.1 and 4 umole/mg/min respectively

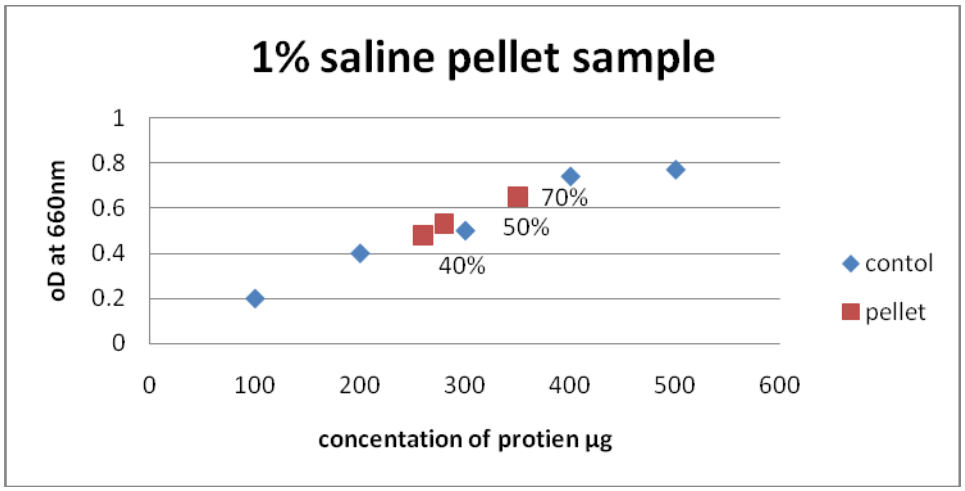

Figure 4: 1% saline pellet graph

Table 5

CONCENTRATION OF AMMONIUM SULPHATE	VOLUME OF PELLET OF 1% OF SALINE CONC (ML)	OD AT 660NM
40%	0.2	0.48
50%	0.2	0.53
70%	0.2	0.65

For 40 %, 50 % and 70 % ammonium Sulphate the Specific Activity of enzyme was found to be 26, 28 and 35 µmole/mg/min respectively

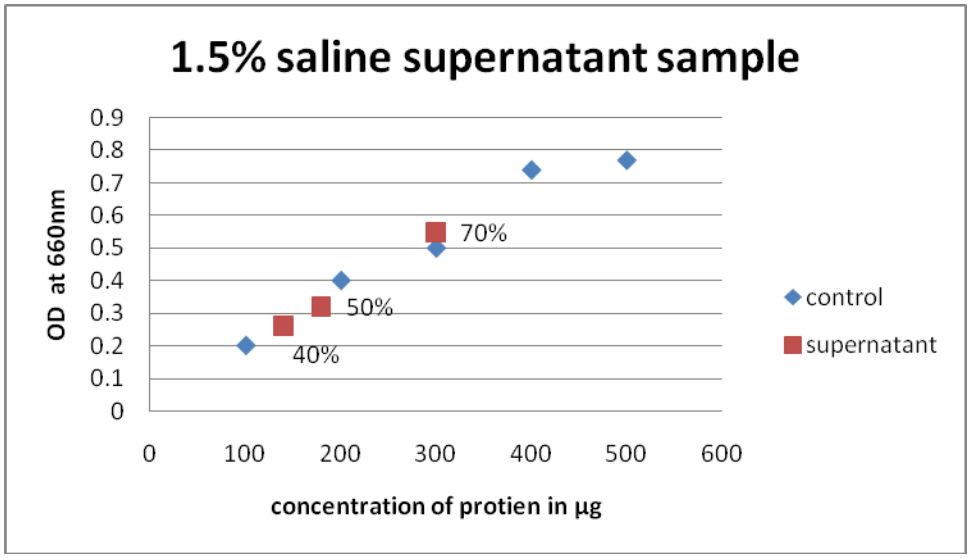

Figure 6: 1.5% saline supernatant graph

Table -6

CONCENTRATION OF AMMONIUM SULPHATE	VOLUME OF SUPERNATANT OF 1.5% OF SALINE(ML)	OD AT 660 NM
40%	0.2	0.26
50%	0.2	0.32
70%	0.2	0.55

For 40 %, 50 % and 70 % ammonium Sulphate the Specific Activity of enzyme was found to be 1.4, 1.8 and 3 µmole/mg/min respectively

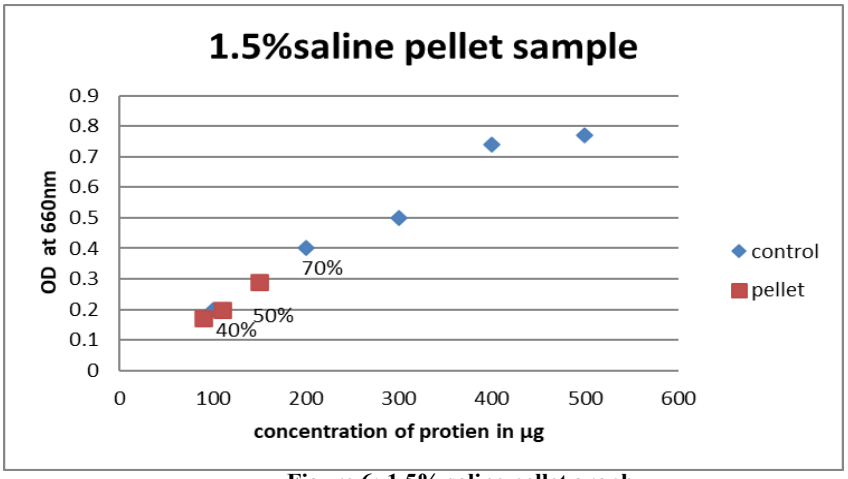

Figure 6: 1.5% saline pellet graph

Table 7

CONCENTRATION OF AMMONIUM SULPHATE	VOLUME OF PELLET OF 1.5% OF SALINE CONC(ML)	OD AT 660 NM
40%	0.2	O.17
50%	0.2	0.20
70%	0.2	0.29

For 40 %, 50 % and 70 % ammonium Sulphate the Specific Activity of enzyme was found to be 9, 11 and 15 umole/mg/min respectively

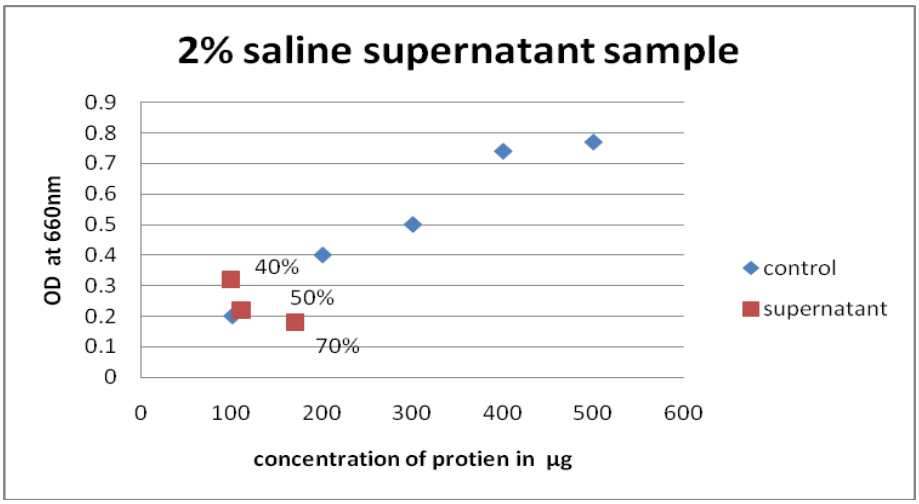

Figure 7: 2% saline supernatant graph

Table 8

CONCENTRATION OF AMMONIUM SULPHATE	VOLUME OF SUPERNATANT OF 2% SALINE CONC(ML)	OD AT B 660 NM
40%	0.2	0.18
50%	0.2	0.22
70%	0.2	0.32

For 40 %, 50 % and 70 % ammonium Sulphate the Specific Activity of enzyme was found to be 0.98, 1.1 and 1.7 µmole/mg/min respectively

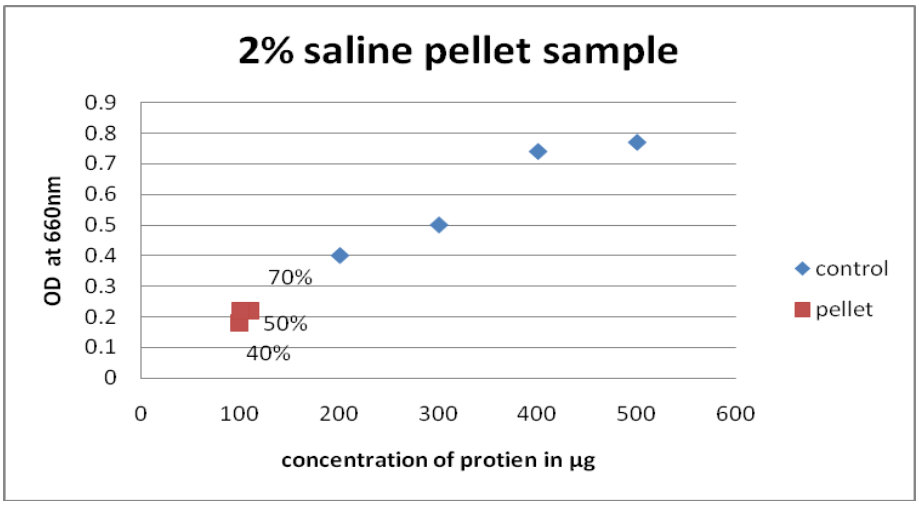

Figure 8: 2% saline pellet graph

Table 9

CONCENTRATION OF AMMONIUM SULPHATE	VOLUME OF PELLET OF 2% SALINE CONC(ML)	OD AT 660 NM
40%	0.2	0.18
50%	0.2	0.22
70%	0.2	0.22

For 40 %, 50 % and 70 % ammonium Sulphate the Specific Activity of enzyme was found to be 9.9, 11 and 10 umole/mg/min respectively

The production of lipase from *Aspergillus niger* fungi showed maximum enzyme activity that was highly significant, cultured in solid state fermentation using agro wastes such as gingelly oil cake and wheat bran. The *Aspergillus niger* culture used the agro wastes as substrate and was highly bound to the substrate which acted as a surface for their growth.

The obtained culture after 72 hours was homogenized using 0.5%, 1%, 1.5% and 2% saline solution. Homogenized suspension was allowed to centrifuge, the obtained supernatant and pellet were used as enzyme source for estimation process. From the assay Procedure and Protein Estimation process that was conducted to obtain partially purified Lipase using chilled 40%, 50% and 70% Ammonium sulphate as precipitating agent.

Estimation was done using both pellet and supernatant, where in maximum enzyme activity was found in pellet. It is known that the activity of enzyme was maximum in 1% saline supernatant and pellet comparatively.

CONCLUSION

Aspergillus niger was the best producer of Lipase enzyme. High enzyme activity was obtained due to cultivation of *Aspergillus niger* in solid state fermentation using agro industrial wastes such as wheat bran and gingelly oil cake as substrate for the production of lipase enzyme. Hence, this method of producing partially purified lipase

using Solid State Fermentation can be done in order to obtain lipase in large scale and also for industrial purposes.

REFERENCES

[1] Vanleeuw E, Winderickx S, Thevissen K, Lagrain B, Dusselier M, Cammue BP, Sels BF. Substrate-specificity of *Candida rugosa* lipase and its industrial application. ACS Sustain Chem Eng. 2019; 7(19): 15828–44. 24.

[2] Badgujar KC, Bhanage BM. Lipase immobilization on hyroxypropyl methyl cellulose support and its applications for chemo-selective synthesis of β-amino ester compounds. Process Biochem. 2016; 51(10): 1420–33.

[3] Sadaf A, Grewal J, Jain I, Kumari A, Khare SK. Stability and structure of *Penicillium chrysogenum* lipase in the presence of organic solvents. Prep Biochem Biotechnol. 2018; 48(10): 977–82

[4] Ito, T., H. Kikuta, E. Nagamori, H. Honda, H. Ogino, H. Ishikawa and T. Kobayashi, 2001. Lipase production in two-step fed-batch culture of organic solvent-tolerant *Pseudomonas aeruginosa* LST-03. J. Biosci. Bioeng., 91: 245-250.

[5] Chisti, Y., 1999. Solid Substrate Fermentations, Enzyme Production, Food Enrichment. In: Encyclopedia of Bioprocess Technology: Fermentation, Biocatalysis and Bioseparation, Flickinger, M.C. and S.W. Drew (Eds.). Vol. 5, John Wiley and Sons, New York, USA., ISBN-13: 9780471138228, pp: 2446-2462.

[6] Salihu A, Bala M, Alam MZ (2016) Lipase production by *Aspergillus niger* using sheanut cake: an optimization study. J Taibah Univ Sci 10: 850–859

[7] Salihu A, Bala M, Bala SM (2013) Application of Plackett-Burman experimental design for lipase production by *Aspergillus niger* using shea butter cake. ISRN Biotechnol 2013: 1–5.

[8] Fleuri LF, de Oliveira MC, de Lara Campos Arcuri M, Capoville BN, Pereira MS, Okino Deglado CH, Novelli PK (2014) Production of fungal lipases using wheat bran and soybean bran and incorporation of sugarcane bagasse as a co-substrate in solid-state fermentation. Food Sci Biotechnol 23:1199–1205.

[9] Edwinoliver NG, Thirunavukarasu K, Naidu RB, Gouthaman MK, Nakajima K, kamini NR (2010) Scale up of a novel tri-substrate fermentation for enhanced production of *Aspergillus niger* lipase for tallow hydrolysis. Bioresour Technol 101:6791–6796.

[10] Dayanandan A, Ravi SHV, Shanmugavel A, Gnanamani A, Rajakumar GS (2012) Solid state bioprocessing for scale up of *Aspergillus tamarii* MTCC5152 lipase and its degreasing effect on cow hide. Indian J Sci Technol 5:2978–2983.

[11] Burkert, J.F.M., Maugeri, F. and Rodrigues, M. I., Optimization of extracellular lipase production by Geotrichum sp. using factorial design, Bioresource Technology, 91, p. 77-84 (2004).