Recovery of Lipase from Overheated Soybean Meal (waste) using *Aspergillus Oryzae*.

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ABSTRACT

In the present study an attempt was made to utilize Overheated Soybean Meal (waste) for the production of lipase by solid state fermentation (SSF) using *Aspergillus Oryzae* (NCIM No. 1212).The study indicated, Overheated Soybean Meal (waste) from Solvent Extraction Industry was suitable for the production of lipase. The effect of varying particle size, pH, moisture content, extraction temperature incubation time and the Lipase activity was investigated during this study.

The study confirmed the exploitation of waste material for the production of value added products which provide support to industries in commercialization of products.

Key words: Solid State Fermentation, Overheated Soybean Meal (waste), Lipase.

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INTRODUCTION

Solid substrate cultivation (SSC) or solid state fermentation (SSF) is envisioned as a prominent bio conversion technique to transform natural raw materials into a wide variety of chemical as well as bio-chemical products [1]. Socio-economic applications of solid state fermentation offer the potential of significant raising standards with only a low technology input requirement [2].

Optimization of SSF conditions is critical in supporting the growth of microorganisms and maximizing the production yield. Key parameters for optimization of solid state fermentation include the carbon and nitrogen sources, compatibility of strains and substrates, initial pH of the growth medium, incubation temperature and period, aeration, mixing, moisture content, and water activity in the substrate [3, 4].

SSF uses agro-industrial waste as support and/or carbon source for production of various value added products, such as single cell protein, industrial enzymes, secondary metabolites, and fine chemicals [5].

Enzymes have application in a variety of areas including food biotechnology, environment, animal feed, pharmaceutical, textile, paper and others technical and chemical industries. Due to the large industrial application and significant cost, there is a necessity to develop processes able to minimize the production costs [6].

After the proteases and amylases, lipases are considered as the third group in volume of sales, moving billion of dollars, showing their application versatility which makes them especially attractive for industrial applications [7,8,9].

Lipases (triacylglycerol acylhydrolases, E.C. 3.1.1.3) are ubiquitous enzymes of considerable physiological significance and industrial potential. Lipases catalyze the hydrolysis of triacylglycerols to glycerol and free fatty acids. Lipase also can be used to accelerate the degradation of fatty waste [10] and polyurethane [11].

The lipases production by SSF on different substrates combined to the large variety of fungi available has already been reported in many studies, as well as the advantages of this fermentation technique when compared to the submerged fermentation [12,13]. In this sense, it is important to mention that the use of agro- industrial residues as substrates in the production of lipase by solid-state fermentation could significantly reduce the final price of the enzyme and also add value to low cost materials on the market [14].

There have been many reports on lipase production by SSF using solid substrates by different species of Aspergillus. *Aspergillus oryzae* produce enzymes such as hemicelluloses, hydrolases, pectinases, lipases, and tannases [15]. Aspergillus oryzae produces at least three extracellular lipolytic enzymes, L1, L2, and L3 [16].

Oil-seed cakes are rich in protein and are recognized as being good food supplements, and some have been used for feed applications in poultry, fish and pig production. They also add value to various biotechnological processes such as the production of enzymes, antibiotics and mushrooms by fermentation [17].Soybean meal as an excellent substrate for lipase production by *Botryosphaeria ribis* EC-01, did not require supplementation with nutrients to increase lipase activity when grown by submerged fermentation [18].Soybean bean meal, defatted soybean meal and soybean protein (all at 4 %) produced lipase by Penicillium camembertii Thom PG-3[19].

The aim of this work was to evaluate the ability of *Aspergillus oryzae* for lipase production by SSF from overheated soybean meal which has been rejected due to lowering of the protein content and becomes waste.

MATERIALS AND METHODS

Microorganism and Inoculum:

Aspergillus oryzae is a fungus widely used in traditional Japanese fermentation industries, including soy sauce, sake, bean curd seasoning and vinegar production. *Aspergillus oryzae* is

known to have prominent potential for the secretory production of various enzymes [20]. *Aspergillus oryzae* NCIM No.1212 was maintained on potato dextrose agar (PDA) slants of pH 5.6 at 4^{O}C.

After tray drying and grinding the substrate was prepared to suitable mesh size by screening. 10 gm each of above grinded substrate taken in different 250 ml conical flask or petriplates was moistened with 15 ml phosphate buffer (7.2 pH) each. Moistened substrate was taken in to autoclave and sterilized for 15 minute at 121^{O}C for proper cooking of the substrate and to increase its amenability for microorganisms.

Aspergillus oryzae NCIM No.1212 spores were transferred aseptically and separately to potato dextrose broath, it is a 100 ml conical flask containing 50 ml of sterilized inoculum medium (sterilized at 121^{O}C for 15 minutes) in laminar air flow. The flask was then kept in incubator at 37^{O} C for 48 hrs. The homogenous spores suspension (10^{6} –10^{7} spores / ml) was used as inoculums [21,22]. Autoclave was used for sterilizing glass wares and media to avoid contamination of undesired microorganism.

Solid State Fermentation:

The substrate used was overheated soybean meal from a same batch and was collected from Maharashtra Oil Extraction Pvt. Ltd., Dhule (Maharashtra, India).The meal is the residue of soybean seed after solvent oil extraction. The substrate was pre-characterized as Oil Content, 0.8%, Moisture Content 7.8%, Protein Content 42%, Fiber Content 9.4%, and Ash Content 2.4%.

After sterilizing the substrate, the substrate was cooled to room temperature. The substrate of 10 gm in petriplate and 15 gm in conical flask of 250 ml were added with the inoculum of 30 % (W/V) in the laminar air flow with the help of sterilized pipette.

Aspergillus oryzae NCIM No.1212 were inoculated on overheated Soybean Meal (waste). After inoculation, the flask and petriplates were then incubated at 37^{O}C for 2 days. The SSF media flasks and petriplates were gently shaken after every 12 hrs for uniform mixing of the substrate and micro organism.

Enzyme Extraction:

The fermented overheated Soybean Meal samples were extracted with 1:10 (W/ V) of 0.1 M sodium phosphate buffer of pH 6.9 was added to each conical flask. The fermented substrate was first taken in 250 ml conical flask in laminar air flow and then buffer was added. The flask was shaken at 150 rpm for 60 minute and material was filtered through muslin cloth or was filtered through whatman filter paper 1. Filtrate collected was centrifuged at 1000 rpm for 10 minutes at room temperature. Supernatant was carefully collected and used as crude enzyme extract for determining protease and lipase activity [23].

Lipase Estimation:

Lipase catalyzes the hydrolysis of triacylglycerols to free fatty acids and glycerol. Lipase activity was determined by the release of fatty acids in the solution which cause decrease in the pH and the rate of the reaction. The librated free fatty acid at different enzyme concentrations was titrated with 0.05 N NaOH. $CaCl_2$ was used as emulsifying agent to increase the surface area and

to decrease the surface tension, thus the oil drop is effetely attacked with the enzyme. Different sets were prepared, using olive oil as substrate and hydrolysis activity was studied. The liberated fatty acid was titrated with NaOH noting the time of the titration (not exceeding) 10 min [24, 25, 26].

Parameters:

The effect of varying particle size, pH, moisture content, extraction temperature incubation time and the Lipase activity was investigated during this study.

RESULTS AND DISCUSSION

Effect of Particle Size on Enzyme Activity

Effect of particle size on Lipase activity was studied by taking Overheated Soybean Meal (waste) samples as substrate. After grinding the substrate the substrate of different particle size from 0.075 mm to 0.850 mm was taken to study the effect of particle size on enzyme activity. Sieve shaker was used to separate the substrate particles of different size. Sieves of different mesh size arranged in a decreasing order of mesh size as 0.850 mm, 0.600 mm 0.425 mm, 0.212 mm, 0.075 mm were mounted on a vibrator.

Substrate of each particle size was taken in conical flask (250 ml) and SSF was carried out for 48 hours at 37^{O}C. The crude enzyme was extracted; a reading of each particle size was recorded for enzyme activity. The graph of mean reading of enzyme activity (shown in percentage) against particle size is shown in figure 1. It has been observed that particle size 0.212mm provide better respiration/aeration and digestion efficiency due to small surface area. In contrast, a large substrate particle or lumps may result in interfere with microbial respiration/aeration and poor digestion of raw material and therefore result in poor growth and enzyme production.

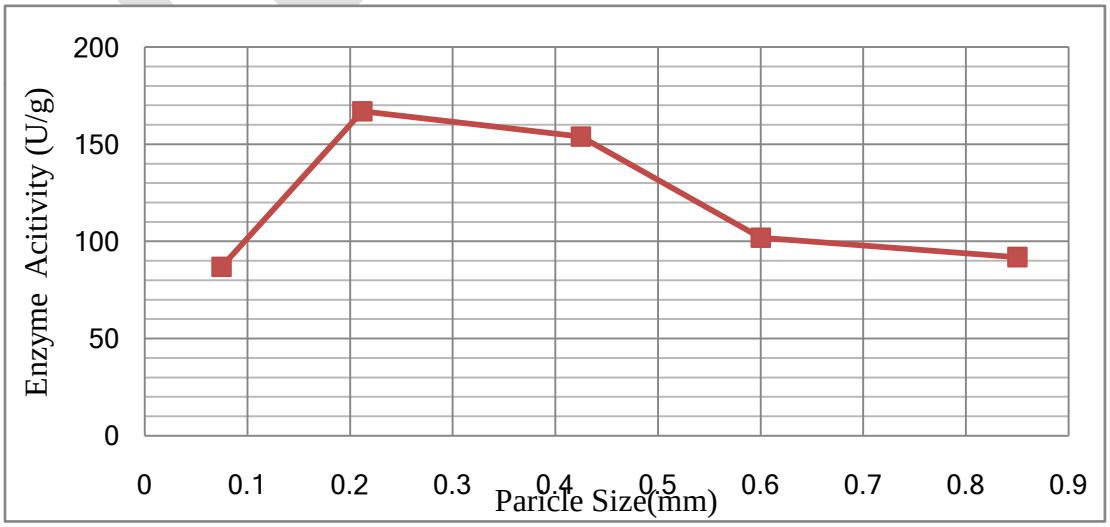

Figure 1: Effect of Particle Size on Enzyme Activity

Decrease in particle size from 0.850 mm to 0.212 mm shows increase in Lipase activity however, further decrease in particle to 0.075mm shown decrease in Lipase activity. Optimal activity of 167 U/g was seen at 0.212mm particle size.

To investigate the effect of extraction pH, incubation pH, incubation temperature, extraction temperature, incubation period, moisture content, particle size 0.212 mm was used for solid state fermentation.

Effect of pH on Enzyme Activity

SSF was performed to study the effect of incubation pH on enzyme activity. A moistened overheated Soybean Meal (waste) had an initial pH of about 6.2. Phosphate buffer (pH 7.2) was used to adjust the initial pH to values greater than 6.2 in separate fermentations. For lipase activity, increase in pH from 6 to 8 increases enzyme activity, further increase in pH decreases activity. Optimal activity of 183 U/g was observed at pH 7.2.

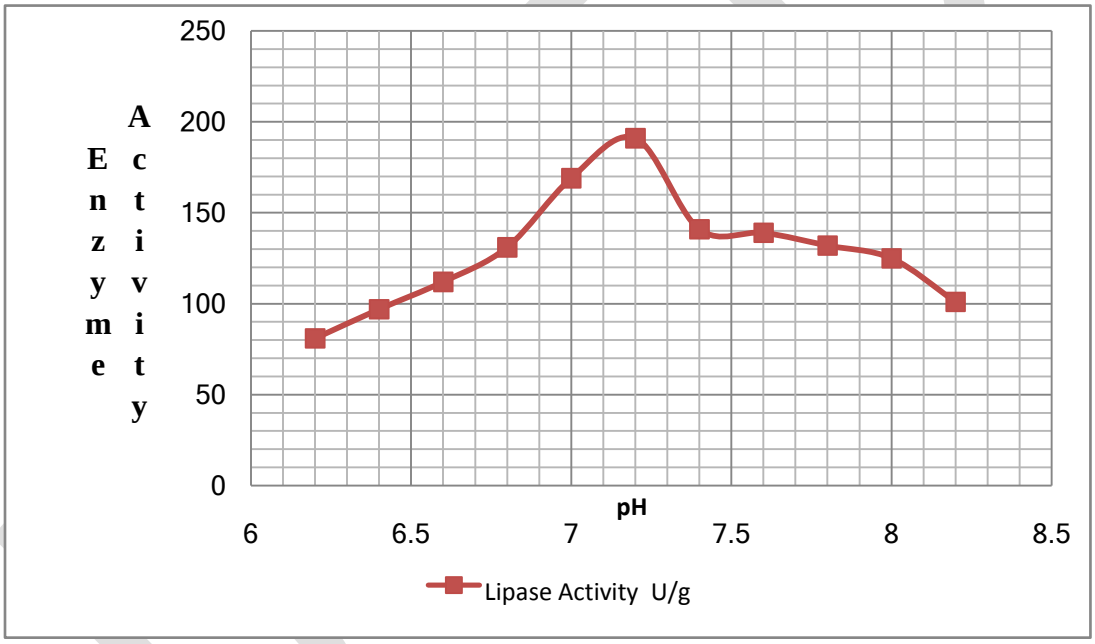

Figure 2: Effect of pH on Enzyme Activity

Effect of Moisture Content on Enzyme Activity

Initial moisture contents of the substrate are known to critically influence mold growth and enzyme production. The impact on physicochemical properties of the substrate affects enzyme production. Presence of water in the substrate makes the nutrients more easily accessible for mold growth. Too much water adversely affects oxygen diffusion in the substrate. In this work, initial moisture contents of the substrate were adjusted to 30, 35, 40, 45, 50, 55 and 60% in separate experiments before inoculation with spores. According to results the optimum initial moisture level was about 45% and the Lipase activity was observed as 191U/g.

Moisture levels much above 45% reduced enzyme production as the substrate became waterlogged, this may be because high moisture content reduces porosity of substrate, which causes particles to stick together and adversely impacts oxygen transfer to the mold. In contrast, a low moisture level reduces water activity to levels that are not conducive to supporting good fungal growth.

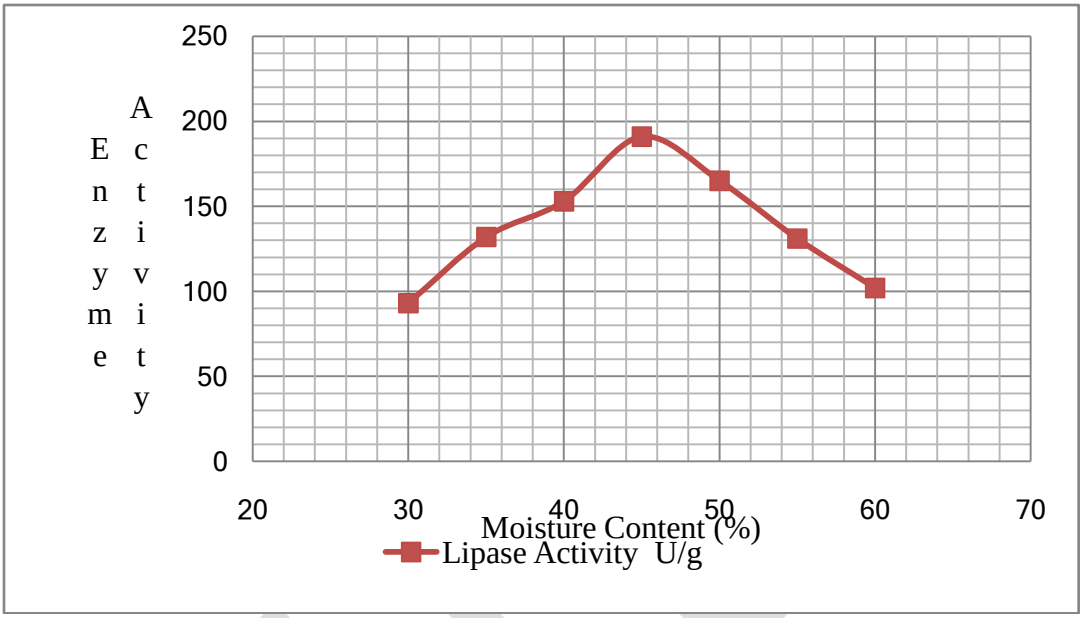

Figure 3: Effect of Moisture Content on Enzyme Activity

Effect of Extraction Temperature on Enzyme Activity

SSF was performed to study the effect of extraction temperature on enzyme activity. Temperatures used were in the range of 25^{O}C to 50^{O}C. After extraction of enzyme, activity was measured at different temperature. The graph of mean readings of enzyme activity (shown in percentage) against extraction temperature explains as the enzyme activity increases with increase in temperature to a maximum and then declines.

At increasing temperature more molecular interaction increases the reaction rate but at elevated temperature, disarray leads to the inactivation of enzymes.

Increase in temperature from 25^{O}C to 35^{O}C shown increase in Lipase activity, whereas onwards 35^{O}C declining in the enzyme activity was observed. The optimum Lipase activity was obtained as 21.2 U/min at 35^{O}C.

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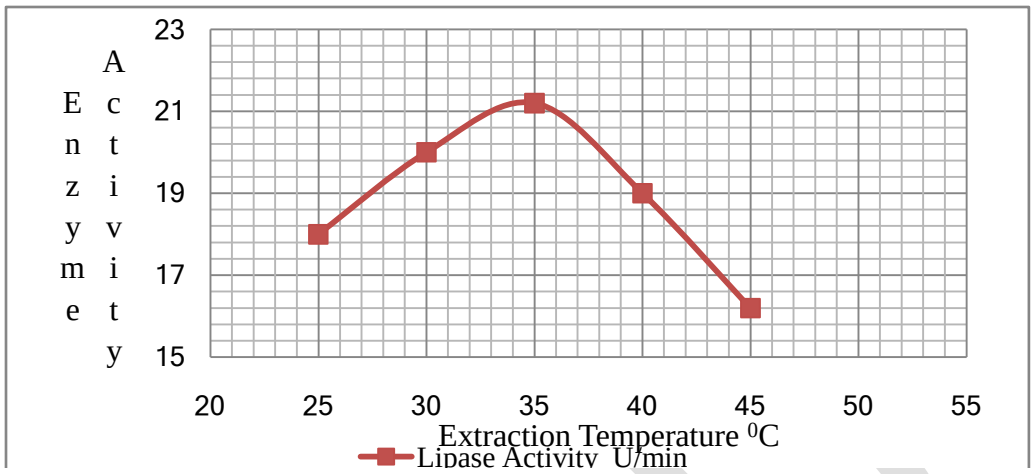

Figure 4: Effect of Extraction Temperature on Enzyme Activity

Effect of Incubation Time on Enzyme Activity

SSF was performed by varying incubation period from 2 to 7 days at 37^{O}C. Increase in incubation period from 2 days to 4 days increases Lipase activity, whereas increase in incubation period from 5 days and above shown decrease in Lipase activity. The optimal activity of 181U/g was observed at 96 hours.

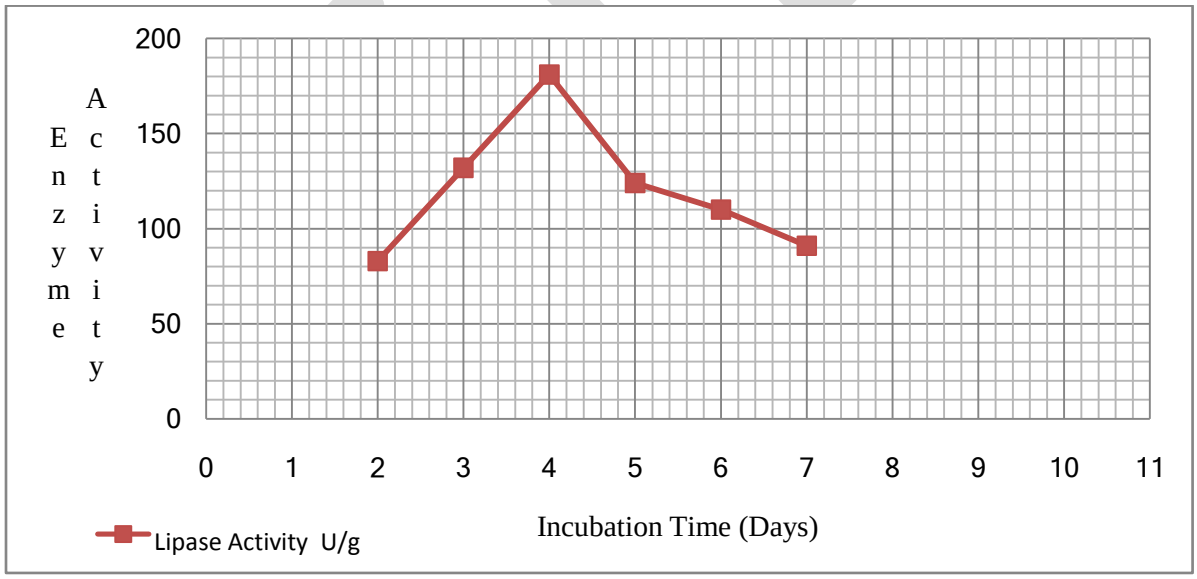

Figure 5: Effect of Extraction Temperature on Enzyme Activity

Determination of Lipase Activity

A Reaction progress curve by plotting the quantity of fatty acid liberated over the time of reaction determines the activity of the lipase. From the slope of the linear portion, the reported Lipase Activity was found as 0.815 µM/min.

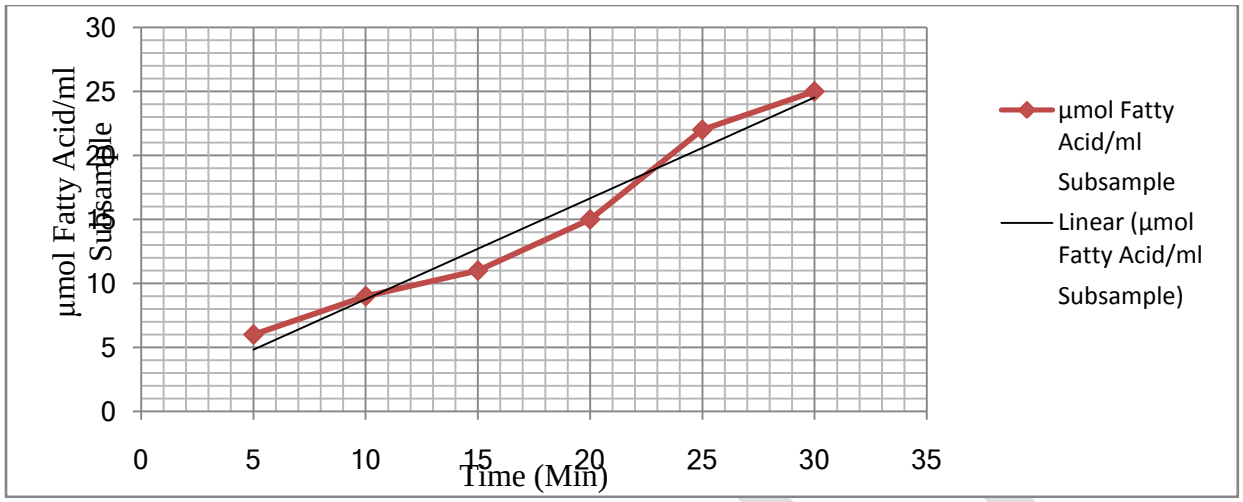

Figure 6: Determination of Lipase Activity

CONCLUSION

SSF has been usually exploited for the production of value-added products. The exploitation of waste material for the production of value added products provide support to industries in commercialization of products. Recovery of value added products from waste biodegradable material is useful for many industrial purposes; recovery of enzymes is one of the alternatives for effective recycling.

Overheated Soybean Meal (waste), has been observed as excellent substrate for lipase production which did not require supplementation with nutrients to increase lipase activity. The maximum activity of lipase produced by *Aspergillus oryzae* NCIM No.1212 on Overheated Soybean Meal (waste) for particle size (167 U/g for 0.212mm), incubation pH (183 U/g for pH 7.2), moisture content (191 U/g for 45%) extraction temperature (21.2 U/min at 35^0C), incubation period (181 U/g on 4^{th} day), and lipase activity (0.8154 µM/min) was recorded.

The results indicate the suitability of agriculture and food industry Overheated Soybean Meal (waste) as solid substrate for large scale production of lipase. Maximum utilization of this waste can also contribute to efficient solid waste management.

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