

# *Solanum tuberosum* peel waste: an attractive substrate for *Aspergillus sp.* CP to produce glucohydrolase under solid state fermentation

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## Abstract

Filamentous fungi are being selected for the production of industrial enzymes as they have enormous capacity of production, on low cost solid substrates. Glucohdrolase is one of the industrial enzymes produced by *Aspergillus niger*. It hydrolyzed alpha 1, 4 and beta 1,6 glycosidic linkage, yield glucose by hydrolyzing polysaccharides from non-reducing end. Major utilization of glucohydrolase is in starch and beverage industry for the preparation of high fructose corn syrup.

The present study is focused to utilize low cost, easily available potato peel waste as substrate for the production of glucohydrolase. Solid state fermentation optimization was carried out by providing suitable physiochemical parameters results in highest enzyme production i.e. 12333 units/ml/min. Dry potato peels 20 grams per fermentation flask, particle size 2mm, was moisten with a fermentation medium containing soluble potato starch 10g/L, peptone1g/L, yeast extracts 2g/L and MgSO<sub>4</sub>.7H<sub>2</sub>O 0.5g/L, (pH 5) with 50% of final moisture content. Fermentation was started with the inoculum of 7.5x10<sup>4</sup> spores per flask and fermentation was carried out at 20°C for 5 days.

**Keywords:** Glucoamylase, SSF, Potato peel, *Aspergillus*

## INTRODUCTION

The potato plant is scientifically known as *Solanum tuberosum*; it is a short herbaceous plant with underground stem called tuber, contains a lot of starch. It is the fourth major crop utilized by human all around the world (Stearns *et al.*, 1994; Mattila and Hellstrom, 2007). Processed potato products demand is continuously increasing and also result in a significant amount of processing waste (FAO, 2008; Schieber and Aranda, 2009). Potato peels consist of the major portion of waste and contain a considerable content of carbohydrate, protein, vitamin C, ferritin, riboflavin, alkaloids (Mäder *et al.*, 2009). Potato peel waste is being used for the production of value-added products like enzymes, bio-surfactants, antioxidants ethanol, and certain polysaccharides (Liang and McDonald, 2014; Amado, 2014).

Glucoamylase (EC 3.2.1.3) is exo-enzyme of extraordinary significance for saccharification of starchy materials and other related oligosaccharides. Glucoamylase successively hydrolyzes 1,4-alpha glycosidic bond from the non-reducing end of starch and other related polysaccharides and release free glucose, which then used as a feedstock for the process of fermentation (Gupta *et al.*, 2003; Norouzzian *et al.*, 2006). Industrial production of glucoamylase has been performed by the fungi, *Aspergillus niger* (Wang *et al.*, 2006). Glucoamylase has different applications in significant areas of food processing, animals feed, fermentation biotechnology, paper making, fabric texture, grain hydrolysis for the alcohol industry (Zambare, 2011).

The production of glucoamylase from different *Aspergillus* species including *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus oryzae*, *Aspergillus terreus* and *Aspergillus awamori* (Negi and Banerjee 2009; Varalakshmi 2009; Biesebeke *et al.*, 2005; da Silva and Peralta 1998; Ghose *et al.*, 1990) have been reported previously. *Aspergillus* and *Rhizopus* are extensively utilized for the industrial production of glucoamylase (Zambare, 2011). Both of above-stated molds were considered an ultimate

applicant for industrial production because of their rich nature, non-fastidious requirements, plentiful amount of production prospective, 100% glucose yield, extracellular secretion, have low transglycosylation action (Mertens and Skory, 2007).

SSF offer increases productivity on easier and more affordable raw materials, after the optimization of physiochemical factors (Yazaid *et al.*, 2017). Potato peel waste as a solid substrate also performs the role of the physical structure that supports the growth of microorganisms in addition to the rich nutrients supply (Gebrechristos and Chen, 2018). Glucohydrolase production in SSF has been reported by Morita and Fujio, 2000; Wang *et al.*, 2006; Sun and Zhang, 2007; Zambare, 2011, and Onofre *et al.*, 2011).

## MATERIAL AND METHODS

### Screening and Identification of Glucohydrolase Producer

Initial isolation and identification of *Aspergillus* were done by colony morphology and microscopic characteristics. Pure cultures were maintained on Potato Dextrose Agar (PDA) at 4°C and were repeatedly subculture after 30 days. Isolated strains were screened for amylase production on starch agar medium; g L<sup>-1</sup>: (Peptone 5.0; Yeast extract 1.5; Sodium chloride 5.0; Starch 10.0; and Agar Agar 20.0). Hydrolytic zones were visualized using Lugol's iodine solution. Positive cultures was further incubated in fermentation medium containing; g L<sup>-1</sup>: (Potato starch 10.0; Yeast extract 2.0; Peptone 1.0; and MgSO<sub>4</sub> 0.5, pH 5.0). After incubation at 30°C for 04 days, culture broth filtered through Whatman filter paper No. 1 and collected cell-free filtrate was used for the estimation of glucohydrolase produced.

### Selection of Fermentation Mode

To produce high titer of glucohydrolase, the mode of fermentation was selected by comparing submerged fermentation with solid state fermentation. Submerged fermentation was carried out as described above and Solid state fermentation was started with 10grams of potato peels as a substrate in individual Erlenmeyer flask (250ml) and moistens with 10ml of fermentation medium. The control flask containing potato peel was moistened with the same volume of distilling water. The flasks were then autoclaved, and after that, each flask was inoculated with 1.0ml of spore suspension. Fermentation was carried out for five days under the static condition at 35±2°C.

### Recovery of Glucohydrolase

After fermentation, glucohydrolase was recovered by re-suspending the solid substrate after the addition of 25mM sodium acetate buffer, pH 4 (50ml) of and vortex for half an hour on a magnetic stirrer. The extracted solution was filtered through filter paper (Whatman No.1) to obtain cell-free filtrate. Glucohydrolase activity was estimated in the cell-free filtrate.

### Determination of Glucohydrolase activity

Glucohydrolase activity was determined in 1 ml of 1% potato starch solution in 25mM sodium acetate buffer pH 4.0 with 0.1ml of enzyme solution. The reaction was carried out for 5 minutes at 50°C, and then after 5min, the reaction was stopped by boiling for 5minutes. The amount of glucose formed was determined by Glucose oxidase (GOD/POD) method using a commercially available glucose kit. One unit of enzyme is defined as the enzyme used to produce one micromole of glucose formed per minute per ml in standard assay condition (Trinder, 1969).

### SSF Substrate Preparation

Potato peels were used as a substrate. They were obtained from the local market. For potato peel, the substrate was washed thoroughly with tap water, air dried and finally, oven dried at 70°C for three days, ground with the help of mortar and pestle, 2mm of particle size were obtained by sieving with the help of sieve having mesh size 10, stored in poly-ethylene bags at room temperature.

### Inoculum Preparation

Inoculum preparation was done by suspending the spores in 15ml of sterile Tween 80 (0.8%) from 5 days-old culture Petri plate, collected in sterile test tubes (Kheng and Omar 2005). Spore count was determined by Neubauer Chamber, and each 250ml flask was inoculated with 1ml of spore suspension having a concentration of 7.5x10<sup>4</sup> spores/ml for solid-state fermentation.

### Optimization of physiochemical parameters

#### Influence of incubation time on glucohydrolase production

The effect of fermentation time on glucohydrolase production was examined by incubating the flasks for 1, 3, 5, and seven days. Each flask containing 20 grams of substrate and 20ml of sterile fermentation medium were inoculated with 1ml of spore suspension and incubated at 35±2°C in the incubator. The enzyme was extracted and assayed after completion of the designated time interval for fermentation.

#### Influence of pH on glucohydrolase production

The effect of fermentation pH on glucohydrolase production was determined by adjusting mineral medium pH at five different values starting from pH 3 to pH 7. The flasks provided with 20 grams of substrate were moistened with 20ml of sterile fermentation mediums (varying pH) were inoculated with 1ml of spore suspension (7.5x10<sup>4</sup>/ml in Tween 80) and incubated for five days at 35±2°C in the incubator.

### Influence of inoculum size on glucohydrolase production

The importance of inoculum size was studied by inoculating each flask with the following number of spores i.e.  $1.5 \times 10^3$ ,  $7.5 \times 10^3$ ,  $1.5 \times 10^4$ ,  $7.5 \times 10^4$  and  $1.5 \times 10^5$  in each flask. Each flask contains 20g of the substrate with 20 ml of sterile fermentation medium was inoculated with different spore concentration and were incubated at  $35 \pm 2^\circ\text{C}$  for five days.

### Influence of temperature on glucohydrolase production

The impact of fermentation temperature on glucohydrolase production was examined by incubating flasks at different temperatures ranging from 20 to  $40^\circ\text{C}$ . The container containing 20grams of substrate and 20ml of sterile fermentation medium was inoculated with 1ml of spore suspension and incubated for five days at 20, 25, 30, 35 and  $40^\circ\text{C}$  in different incubators.

### Influence of particle size on glucohydrolase production

The influence of particle size of potato peel on glucohydrolase production was investigated by comparing the different particle size, i.e., 0.5, 1, 2, and 3mm. Flasks containing one of the mentioned particle sizes were moistened with 20ml of fermentation medium, inoculated with 1ml of spore suspension incubated at  $35 \pm 2^\circ\text{C}$  for five days.

### Influence of substrate content on glucohydrolase production

The impact of substrate content on enzyme production was tested by varying the amount of potato peel substrate to the mineral medium ratio (w/v). The 250 ml Erlenmeyer flasks containing 5g, 10g, 20g, 30g and 35 grams of the substrate in the respective flask was moistened with the 20ml of fermentation medium inoculated with 1ml of spore suspension and incubated for five days at  $35 \pm 2^\circ\text{C}$  in the incubator.

### Influence of moisture level on glucohydrolase production

The effect of moisture level on glucohydrolase production was evaluated in solid substrate by changing substrate to fermentation media ratio (w/v) in the range of 20% - 60%. The 250ml of Erlenmeyer flasks containing 20g of dry potato peels as substrate and 4.6ml, 8.5ml, 13.5ml, 20ml and 30ml of medium maintaining 20%, 30%, 40%, 50% and 60% moisture levels respectively. After sterilization, flasks were inoculated with 1ml of spore suspension ( $7.5 \times 10^4$  /ml) and incubated for five days at  $35 \pm 2^\circ\text{C}$  in an incubator.

## RESULTS AND DISCUSSION

### Screening and selection of fermentation mode for glucohydrolase production

*Aspergillus* strains were screened for the production of glucohydrolase on starch agar medium, *Aspergillus niger* CP, isolated from rotten capsicum, expressed a clear starch hydrolytic zone around the colony. Quantitative estimation confirmed that *Aspergillus sp.* CP is a hyper producer of glucohydrolase producing 3,211.0 units/ml/min under solid state fermentation then Submerged fermentation (1689 units/ml/min) (Figure 1). Further optimization studies were carried out using *Aspergillus sp.* CP for the production of glucohydrolase under solid state fermentation.

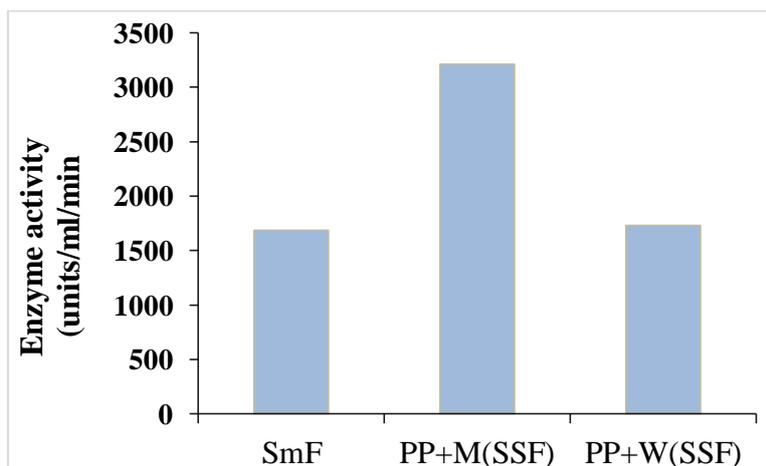


Figure1: Selection of fermentation mode for glucohydrolase production by *Aspergillus sp.* CP. SMF = submerged fermentation, SSF= Solid-state fermentation. PP= potato peel, M= fermentation medium, W= Distil water.

In the present study, potato peels waste shows considerably higher glucohydrolase production, as it is a rich source of carbohydrate and protein. Rapid microbial spoilage of potato peels waste grounds a serious disposal problem in the potato industry and could be solved by utilizing waste as a selective substrate for the production of starch degrading enzymes (Mahmood *et al.*, 2016; Tamilarasan *et al.*, 2010; Schieber and Saldana, 2009). Optimization of glucohydrolase production was performed under SSF using a fermentation medium containing soluble potato starch, organic nitrogen sources yeast extract and peptone, and  $\text{MgSO}_4$ . The previous reports describe the positive impact on enzyme production and secretion by supplementing the solid substrate with an available form of potato starch and yeast extract (Mustaq *et al.*, 2017; Hassan and Karim, 2015; Nwagu and Okolo 2010).

### Influence of incubation period on the production of glucohydrolase

Incubation period plays an integral role in substrate utilization and enzyme production. The effect of incubation period was studied by checking enzyme activities after 1, 3, 5, and seven days of incubation. In our experiment, maximum glucoamylase production (3357 u/min/ml) was obtained on the 5<sup>th</sup> day of incubation (Figure 2). Maximum enzymes production was mostly reported in 5 days, and afterward, enzyme activity declined, which may be due to an increase in acid production as waste or may be due to complete nutrient depletion. It was found that amylase production was highest on the 5<sup>th</sup> day of the incubation period (Gupta *et al.* 2008; Chimata *et al.*, 2010).

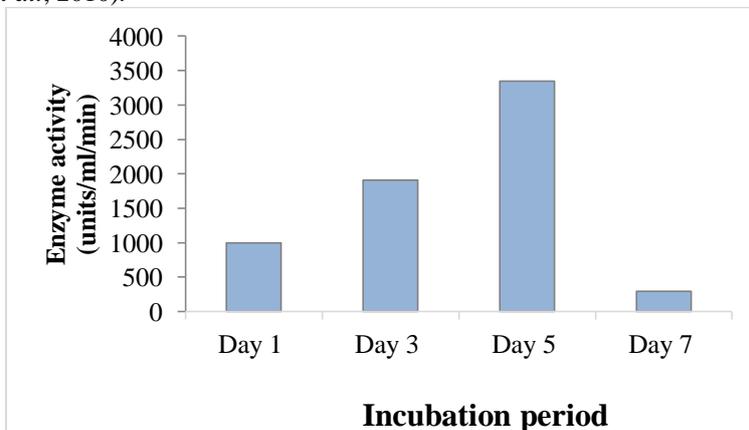


Figure 2: Influence of incubation period on Glucohydrolase production in SSF by *Aspergillus sp.* CP.

### Influence of pH on glucohydrolase production

The effect of pH on glucoamylase activity of *Aspergillus sp.* CP was studied by varying the pH from pH 3 to 7. Results from the graph showed enzymes production was monitored in pH range from pH 4 to 6. At pH five maximum enzyme productions was recorded and gradually decreases with pH 6. It is indicated from the graph that pH 5 is best for maximum enzyme production (Figure 3). Usually, it is desirable to use fungus, which will prefer pH values slightly acidic, to control contaminations with another organism (Kar & Ray, 2008; Mahmood *et al.*, 2016).

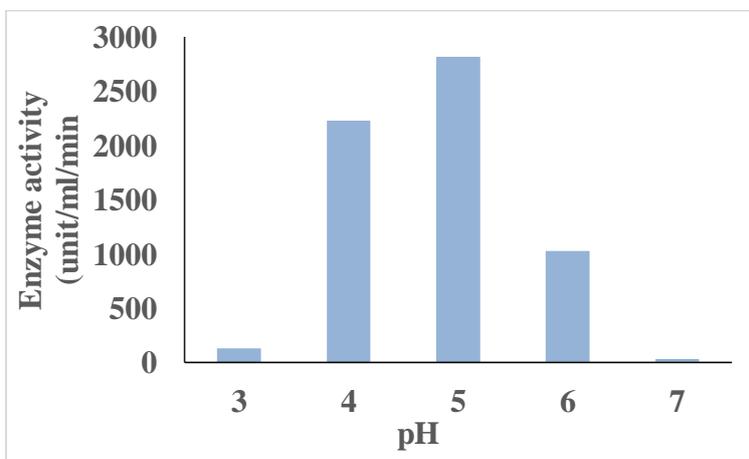


Figure 3: Influence of initial pH on Glucohydrolase production by *Aspergillus sp.* CP.

### Influence of inoculum size on glucohydrolase production

Inoculum concentration is also an essential component in solid state fermentation. The presented results showed that the inoculum size of  $7.5 \times 10^4$  spores gives optimum enzyme production in the 20g of potato peel as substrate (Figure 4). Inoculum size of  $1 \times 10^4$  spores of *Aspergillus niger* USM AI 1 was reported for xylanase production (Kheng and Omar, 2005).

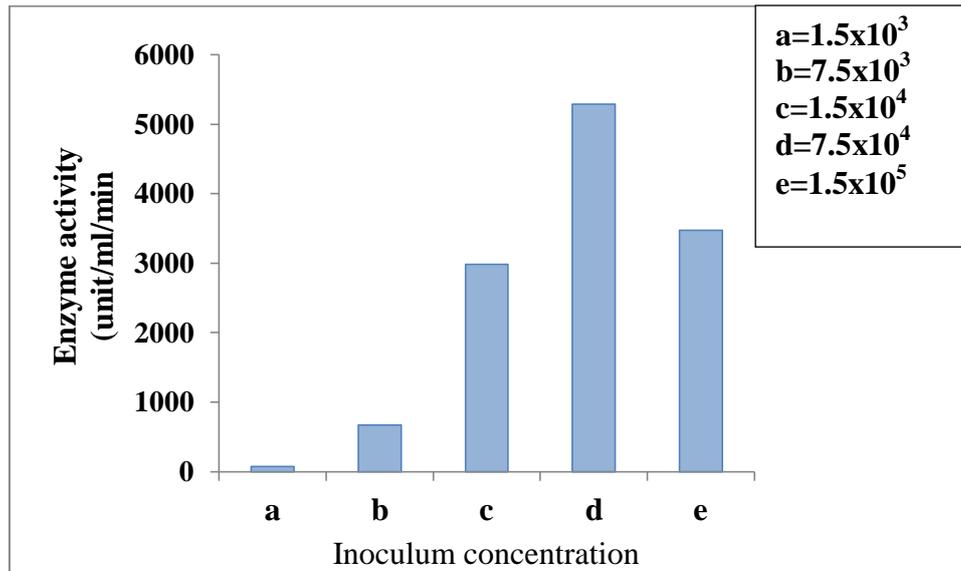


Figure 4: Influence of inoculum concentration on glucohydrolase production by *Aspergillus sp.* CP.

#### Influence of incubation temperature on the production of enzymes

Incubation temperature not only influences the growth of microorganisms but also their biological activities. The effect of temperature on glucoamylase activity was investigated by keeping temperatures under following given range, i.e. 20 to 40°C. The results represent in the graph clearly shows that maximum enzyme activity was found at 20°C. It might be due to the reason that at high temperature, the growth of the fungus was inhibited and hence, enzyme formation was also inhibited (Figure 5). Maximum amyolytic activity of *Aspergillus oryzae* on wheat bran was reported at 30°C in 3days (Sivaramakrishnan *et al.* 2007). Chimata *et al.*, (2010) reported the maximum extracellular amyolytic activity of *Aspergillus sp.* MK07 was on day five at 30°C.

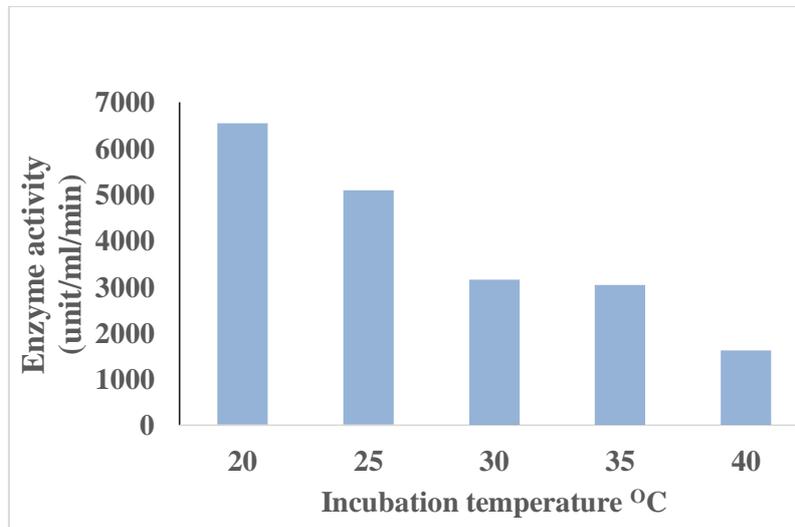


Figure 5: Influence of incubation temperature on glucohydrolase production by *Aspergillus sp.* CP.

#### Influence of particle size on glucohydrolase production

Highest enzyme activity was reported with 2mm particle size, and then enzyme activity gradually decreases with increasing particle size to 3mm-4mm, as less surface area available to the microbial enzyme to be acted upon (Figure 6). Appropriate particle size should provide enough surface area for mycelium growth of fungus and satisfy the oxygen demand (Pandy, 2003).

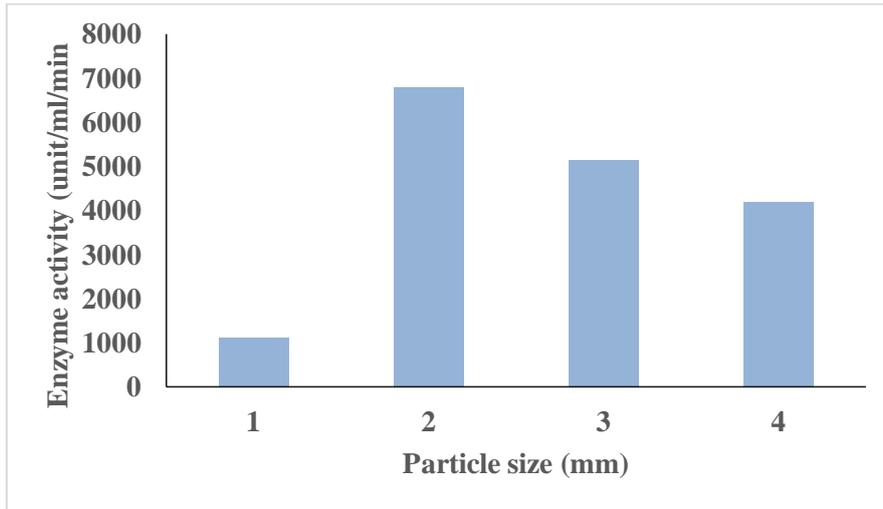


Figure 6: Influence of particle size on glucohydrolase production by *Aspergillus sp.* CP

**Influence of substrate content on glucohydrolase production**

Solid state fermentation is greatly affected by substrate content. The effect of substrate content on enzyme production was evaluated; 20g of substrate per 250 ml of Erlenmeyer flasks (1:12.5) shows the maximum activity of the enzyme (Figure 7). Results illustrate that increase or decrease in substrate mass (20g/flask) drastically decrease enzyme yield, as porosity and aeration were influenced (Bhatti et al., 2007).

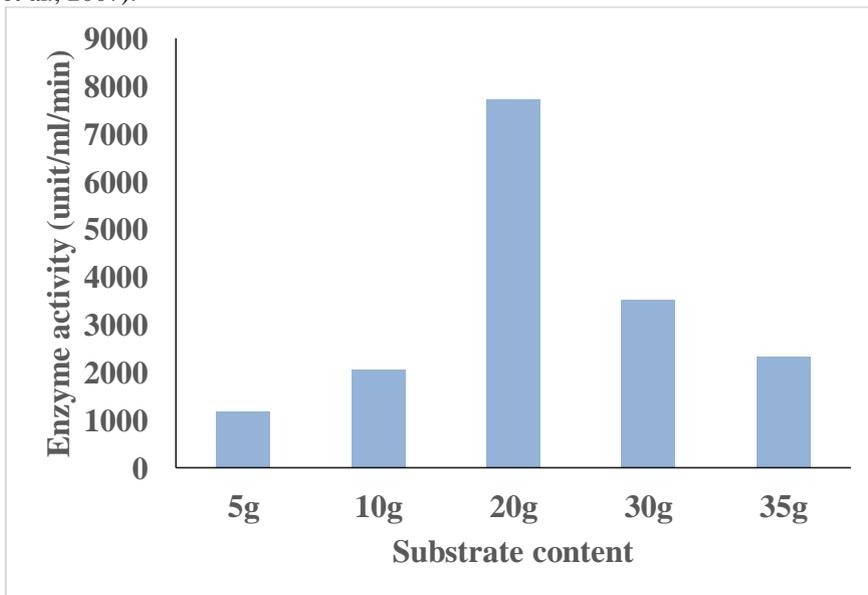


Figure 7: Influence of substrate content on glucohydrolase production by *Aspergillus sp.* CP

**Influence of moisture level on glucohydrolase production**

The results shown that glucohydrolase activity increases with increase in moisture content from 40% to 50%, was observed maximum at 50% and then gradually fall off at 60% moisture content as the substrate became waterlogged and decrease surface to volume ratio (Figure 8). Morita and Fujio, (2000) reported that 45% initial moisture level was optimum for enzyme production by *Rhizopus sp.* MKU 40.

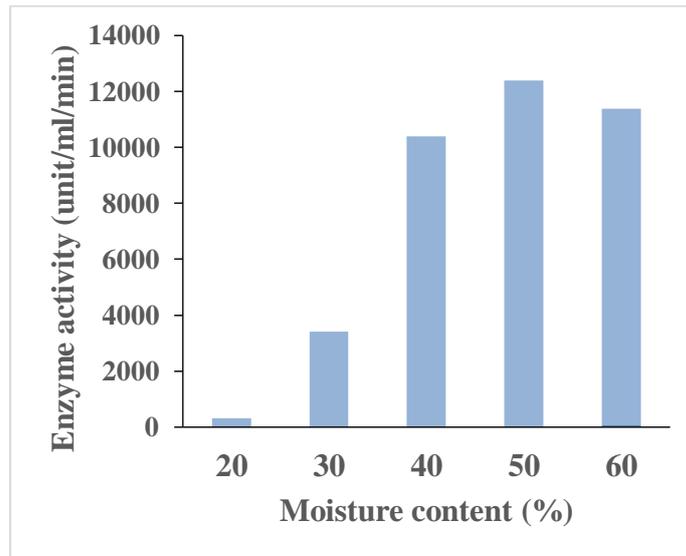


Figure 8: Influence of moisture content on glucohydrolase production by *Aspergillus sp.* CP

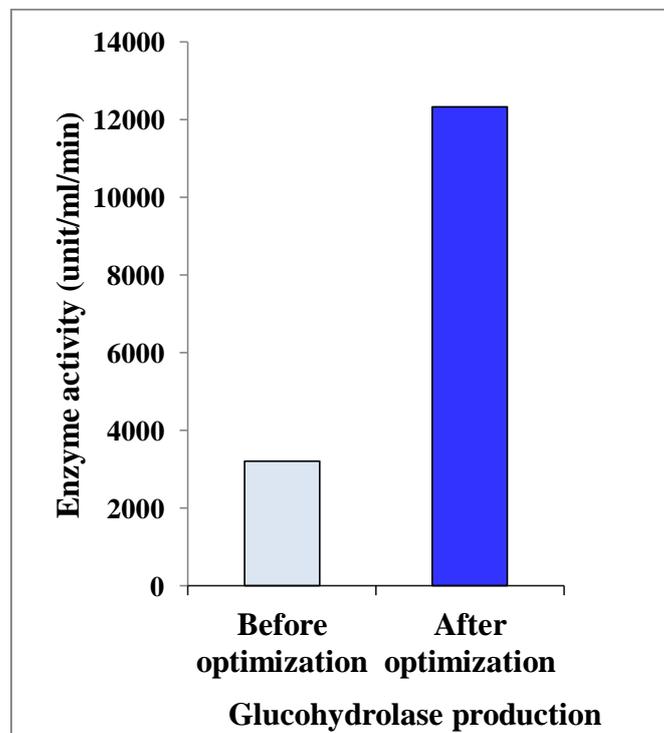


Figure 9: Glucohydrolase production before and after optimization of SSF on potato peel waste by *Aspergillus sp.* CP.

This study, which was aimed to investigate the optimum conditions under solid state fermentation for maximum production of glucoamylase by *Aspergillus* strain CP. Highest enzyme activities, i.e., 12333 units/ml/min, were found when 20 grams of potato peel was used as a substrate with 50% of moisture content having a particle size 2mm.  $7.4 \times 10^4$  spores were optimum inoculum size. Fermentation was carried out at 20°C at pH 5 for five days (Figure 9). Hence, after optimizing all parameters, a threefold increase in glucohydrolase production was observed. Hence, it can be concluded that the utilization of potato peel waste by *Aspergillus sp* CP is an inexpensive, eco-friendly way of enzyme production through solid-state fermentation.

#### CONCLUSION

Based on the above results, filamentous fungus *Aspergillus sp.* CP demonstrates an extraordinary capacity for glucohydrolase production from potato peel waste based on solid-state fermentation. Optimization of process parameters in this study found to be productive and has the potential to be exploited for the production of these biotechnological importance enzymes.

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### CONFLICT OF INTEREST

All authors declare no conflict of interest, financial or other, exists. Authors declare that they respect the journal's ethics requirements.

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