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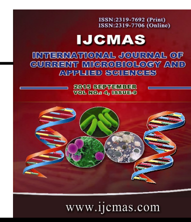

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Original Research Article

Screening and Selection of Fungus for Keratinase Production by Solid State Fermentation and Optimization of Conditions of SSF and Formulation of Low Cost Medium for the Production of Keratinase by *Aspergillus flavus* S125

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Keratinase, Solid state fermentation, *Aspergillus flavus,* Keratinolytic fungus, Feather keratin, Optimum pH

A B S T R A C T

The Solid-state fermentation (SSF) is the growth of organism on moist substrates in the absence of free flowing water. It is more techno-economically important compared with the submerged fermentation. Screening of fungi for keratinase production by SSF was done in two steps – a preliminary study and the detailed SSF study. In preliminary studies all the 42 keratinolytic fungi were subjected to SSF providing arbitrarily selected conditions of moisture level and incubation period *i.e*, solid substrate: moistening solution ratio 1:2 and 5 days of incubation. The fungal isolates found to be keratinolytic on keratin agar medium were screened for keratinase production by solid substrate fermentation.Present study showed that the optimum moisture level of 69.92% presented better enzyme production, above which the production started decreasing.The maximum production was obtained at 4th day of incubation at pH 9 and temperature 55°C. Carbon and Nitrogen sources were found insignificant in enzyme production.

Introduction

Keratin is the protein present in hair, horns, claws, nails, hooves etc. Currently, the term 'keratin' covers all intermediate filament-forming proteins with specific physicochemical properties. The protein chains are packed tightly either in α-chain (α-keratin) or in -sheet (-keratins) structures. A group of proteolytic enzymes hydrolyze insoluble keratins more efficiently than other proteases are called keratinases (Onifade et al., 1998). They are produced mostly by microorganisms.

Keratinases [EC.3.4.99.11] belong to the group of serine proteases capable of degrading keratin. There are many reports on the isolation and identification of keratinases from different microbial sources. Keratinases can be produced by several species of fungi, bacteria and actinomycetes. Microbial keratinase has many industrial applications due to their biochemical diversity and wide applications in tanneries, food industries, waste treatment etc. Further the keratinous wastes are accumulating and

there is a demand for developing biotechnological alternatives for recycling such wastes.

Optimization of conditions of solid state fermentation and formulation of a low cost medium for the production of keratinase by *Aspergillus flavus* S125

Various factors influencing the production of keratinase enzyme by SSF by the selected fungal strain *Aspergillusflavus* S125 were focussed for the study. The factors influencing the enzyme production were experimented one at a time, keeping other factors constant. Once optimization was done with respect to a factor, it was incorporated in the experiment for the optimization of the next factor. It is estimated that, growth media accounts for 30-40% of the production cost in enzyme industries (Kumar *et al.,* 2004). Therefore, the feasibility of enzyme production on low cost fermentable substrates needs to be studied. Thus a medium was designed based on the results and the enzyme production in this medium was studied in detail.

Materials and Methods

Fungal Strains

A total of 42 keratinolytic fungal isolates along with *Chrysosporium keratinophilum* MTCC1367 are subjected to solid substrate fermentation studies.

Preliminary SSF studies

The fungi were subjected to solid state fermentation studies using feather keratin substrate.

Preparation of feather keratin substrate

Large amount of chicken feather was collected from the poultry farm and washed well with chloroform-methanol mixture (1:1, v/v) and finally with distilled water. It was then dried in sunlight, tyndallised at 100˚C for 20 min for five successive days and then powdered.

It was moistened with mineral salt solution in the ratio of 1:2. 10 gm of feather keratin powder prepared was taken in 250 ml Erlenmeyer flasks and mixed thoroughly with 20ml mineral salt solution. The flasks were sterilised by autoclaving at 121˚C for 30 minutes.

Inoculation & Incubation

Spore suspension of the fungal isolates was prepared as described under section A of this chapter. Flasks were inoculated with 2 ml. suspension containing $2x10^8$spores/ml. After inoculation the flasks were incubated at room temperature (30±4˚C) for 5 days in a humidified chamber having relative humidity 70%.

Extraction of enzyme

Phosphate buffer having pH 8 was used for the extraction of culture filtrate. After 4-5 days of incubation, 100 ml. of phosphate buffer (pH 8) was added to each flask. The flasks were agitated for extraction of enzyme in the shaking incubator (Labline) at 200rpm for 1 hour at 30˚C. After I hour the contents were squeezed using muslin cloth and the liquid portion was used as the crude enzyme.

Measurement of dry weight of mouldy substrate

The weight of the mouldy substrate was determined gravimetrically by drying the sample at 100˚C to a constant weight. The enzyme activity was expressed in Units per gram dry weight. (U/gds).

Identification of high yielding isolate

The high yielding isolates were identified based on cultural and morphological characters.

Detailed SSF studies

Seven high yielding strains were subjected to detailed SSF studies with the feather keratin and MSS medium at ratios 1:0.5, 1:1, 1:1.5, 1:2, and 1:2.5. The enzyme assay was done after 3, 4, 5 & 6 days of incubation.

Materials needed for the optimization process are discussed below

Solid substrates

Different types of commercially available substrates were subjected for culturing the fungus like rice bran, wheat bran, wheat powder, coconut oil cake, ground nut oil cake and feather keratin under solid state fermentation. The keratinase enzyme yield was determined.

Particle size

Feather powder was sieved to get particles of different size and utilized for SSF and the production of the enzyme was estimated.

Moisture content, incubation temperature and incubation period

The solid substrate was moistened with different concentrations of mineral salt solution. The initial percentage of moisture content was determined. A portion of the moistened substrate was weighed and dried to constant weight in a hot air oven set at temperature 80˚C.The weight of the dried substrate was also taken. Moisture content of the substrate was calculated as per the formula

% moisture content =

$$\frac{\text{Wt. of the substrate before drying} - \text{Wt. of the substrate after drying}}{\text{Wt. of the substrate before drying}} \times 100$$

Incubation was performed at different temperature (35˚C-55˚C) for different periods of time and the enzyme production was determined.

pH of moistening agents

The moistening solutions (Mineral salt solution) of different pH from 7 to 10 were used and the effect of pH on enzyme yield was determined.

Supplementary carbon Sources

Different carbon sources were added into (MSS) so that the final concentration in the solid substrate media was 2% (w/w) and the enzyme production was determined.

Supplementary nitrogen sources

The effect of nitrogen sources, on keratinase production was studied. The final concentration of nitrogen sources in the solid substrate medium was 2% (w/w).

Media volume: Flask volume

SSF was performed with different quantities of moistened substrates taken in 250 ml. conical flasks and the enzyme production was tested.

Formulation of production medium

Feather substrate: Preparation of feather substrate was done as described under chapter 3 section A.

Preparation of moistening solution

The moistening solution for the feather keratin medium contained (g/l) KH_2PO_4 1.8; K_2HPO_4-6.3; $MgSO_4$-1.0; and $MnSO_4$ 0.1, $FeSO_4$ – 0.1and feather keratin – 10.pH was adjusted to 9.

Culture method and applied parameters

40 ml of the prepared mineral medium with 20gms of feather was distributed in 250 ml Erlenmeyer's flask and was sterilized at 121°C for 20 min. The flasks were inoculated with 4 ml of spore suspension containing $2x10^8$/ml prepared as described under chapter 3 and incubated at 37°C.

Study of feather degradation

During the period of incubation, samples were drawn at regular intervals of 10 days and analyzed to study the progress of feather degradation. The culture filtrate was centrifuged at 3000 rpm for 5 min and the collected supernatant was used to assess the changes in pH, estimation of protein and the enzyme. Percentage of residual substrates also was calculated.

Protein estimation

The amount of protein in the culture filtrate was quantified by the method explained by Lowry *et al.* (1951). Mixed 0.5 ml of sample with 5 ml of freshly prepared alkaline copper sulphate and incubated at room temperature for 10 min.

To this 0.5 ml of Folin-Ciocalteau's reagent was added and incubated at room temperature for 20 min and the absorbance was measured at 660 nm wavelength. The blank was prepared using the same procedure without sample. The protein content was estimated by calibration with the standard graph.

Change in pH

pH of the filtrate of the medium was measured using a pH meter (Systronics) with glass electrode.

Substrate degradation (%)

The undigested residue and cells remaining in the filtrate and was dried. The % substrate hydrolysis was determined by the formula:

$$\frac{\text{Dry wt. of the substrate before drying} - \text{Dry wt. of the substrate after drying}}{\text{Dry wt. of the substrate before drying}} \times 100$$

Result and Discussion

Preliminary SSF studies

The amount of enzymes produced by seven high yielding strains are shown in table 1.

Detailed SSF studies

Result of the detailed SSF studies presented in table 2.

In the detailed SSF studies also, the isolate S125 was found to be the highest yielding one.

The Solid-state fermentation (SSF) is the growth of organism on moist substrates in the absence of free flowing water. It is more techno-economically important compared with the submerged fermentation. Screening of fungi for keratinase production by SSF was done in two steps – a preliminary study and the detailed SSF study. In preliminary studies all the 42 keratinolytic fungi were subjected to SSF providing arbitrarily selected conditions of moisture level and incubation period *i.e.*, solid substrate:

moistening solution ratio 1:2 and 5 days of incubation. In the present study feather keratin was used as the medium for screening and isolation of the fungi. Only a few works have been reported using feather keratin as substrate for screening the fungi.
The fungal isolates found to be high yielding by preliminary SSF studies were identified as *Alternaria* sp. *Aspergillus flavus, Aspergillus fumigatus*, *A. nidulans Crysosporium keratinophilum, Doratomyces microspores* and *Paeciliomyces.* Of these 3 strains *A. flavus, A. fumigatus* and *C. keratinophilum* were high yielding under SmF also. Detailed SSF studies varying the moisture content and incubation period showed that the strain S125 performed best at 5th day of incubation (486.90 U/gds). MTCC strain also presented better yield in 4th day of incubation, but comparatively low (324.45U/gds). The conditions influencing enzyme production by SmF and SSF can be different and it is generally believed that separate screening procedures are necessary for the isolation of strains for submerged and solid substrate fermentation.

The result of the present study also conforms to these observations. Among the 7 high yielding strains under Smf and SSF only 3 were common viz, *A. flavus, A. fumigatus and C.keratinophilum.* Hence it is opined that separate screening procedures should be adopted for the selection of suitable strains for these two types of fermentations. However it was interesting to note that *A.flavus*S125 was the highest yielding strain under both SmF and SSF in the present study. This strain was already identified by molecular methods and was selected for further studies.

Effect of solid substrates

Effects of different solid substrates on the production of keratinase are shown in table 3. Among the different substrates used in solid state fermentation feather keratin rendered better enzyme activity followed by wheat bran.

Effect of particle size

Effect of feather powder of different particle size on the production of keratinase enzyme is shown in the table 4.
Of the different particle size used, particle size 500-710 excelled in keratinase production. However the ungraded feather powder also found equally good.

Effect of Moisture content, temperature of incubation and incubation period

The effects of moisture content, temperature of incubation & incubation period on the production of keratinase enzyme is shown in the table below. % moisture content is given in brackets.

Keratinase production was seen at its peak at incubation period 4 days at temperature 55˚C in the ratio 1:2 (substrate and moistening solution).

Effect of pH of the moistening solution

Effect of pH of the moistening solution on the production of keratinase is given in the figure 1.

Highest keratinase production was recorded at a pH 9 of the moistening agent.

Effect of various supplementary carbon sources on enzyme production

The effect of supplementation of the feather keratin medium with carbon sources on keratinase production is summarised in table 6.

All the carbon supplements (2% w/w) lowered keratinase production. Control itself was found to be the best in keratinase production.

Effect of Nitrogen sources on enzymatic activity

The effects of supplementation of the medium with organic and inorganic nitrogen sources are given in table 7.
All the organic nitrogen sources at 2% w/w tested were found inhibiting the production of keratinase enzyme.

Effect of Inorganic Nitrogen sources on Enzyme production

Inorganic nitrogen supplements also exhibited the inhibitory effect on enzyme production.

Ratio of medium volume and flask volume

The maximum yield of keratinase enzyme was obtained at a ratio of 1:4.5.

Feather degradation and enzyme production

Keratinolytic activity and protein concentration in the culture filtrate during feather degradation.

The production of keratinolytic enzyme was found at its maximum of 784 U/ml at the 40th day when feather was used as substrate. It is evident from the results that there was gradual increase in the keratinolytic activity of the *A. flavus* S125up to 40 days but decline thereafter. A gradual increase in the protein concentration in the culture filtrate was noted till the 40th day of incubation. Afterwards it was also seen to be declined. Keratinase production was observed at its maximum (636-668 U/gds) up to 40th day and a decline thereafter.

Change in pH

The changing pH during feather degradation Keratinase production was obtained at its maximum at 40th day and a visible decline in production was noted afterwards.

Optimization of culture conditions was essential to obtain maximum yield by the selected fungus, *Aspergillusflavus*S125. Solid-state fermentation (SSF) is the fermentation involving solid substrates with enough moisture to support growth and metabolism of micro-organism (Mitchell and Lonsane, 1990; Pandey *et al.,* 2001). In SSF, optimization is highly important because many factors have critical influence on the production of enzymes. Cultivation parameters such pH, temperature, media composition etc. are optimized for maximum enzyme production. Past few years SSF has been shown to be remarkably useful in the industrial production of a myriad of value added products. This method is advantageous over submerged fermentation (SMF) in terms of low production cost, saving water and energy, and less effluent problem.

Present study revealed that among various substrates used for solid state fermentation, feather keratin substrate rendered highest enzyme yield. Keratinous wastes are accumulating in the environment and there is demand for recycling. Different microbes like fungi, bacteria and actinomycetes have been identified and reported to degrade and utilize keratin as they are producing keratinase enzymes. Filamentous fungi are the best studied for SSF due to their hyphae which have the capability to not only grow on the surface of the substrate particles but also to penetrate through them (Pandey *et al.,* 2000). Poultry feather contain alpha or beta keratin which are resistant to degradation. Hydrolysis of such wastes provide beneficial product that could find

their application in several industries e.g., biodegradable films, glues and leather as well as in agriculture as nitrogenous fertilizer for plants.Degradation products of feather have great commercial value since they are useful as supplements for digestible feed for poultry, livestock and fish. Liquid nutrient residues from feather degradation that are high in nitrogen could be used for aquaculture and hydroponics crop. (Ichida *et al.,* 2001). Feathers contain large amount of protein and can also be utilized for the production of biogas. Feathers contain large amount of cysteine, glycine, arginine and phenylalanine. (Baker *et al.,* 1981) and can be used as a cheap and readily available substrate for fungal growth. This could result in a substantial reduction in the costs of enzyme production.

Various physical and chemical factors were found influencing the production of keratinase by the fungus. Present study showed that the optimum moisture level of 69.92% presented better enzyme production, above which the production started decreasing. This may be due to inadequate aeration in the medium or the reduction in the available surface area for the anchorage of cells. The moisture at optimum level is highly essential for maximizing the growth or metabolite production. In the case of low moisture level than the optimum, there might be decreased solubility of nutrients. High substrate moisture results in decreased substrate porosity resulting in lesser surface area for the anchorage of mycelium. A steady increase in enzyme production was observed with the progress in incubation periods. The maximum production was obtained at 4th day of incubation which was not much high when compared with the enzyme production on the 3rd day. Similar type of result was obtained in the case of production of protease by *Myrothecium verrucaria* in solid state cultures using feather meal as substrate in which maximum protease production was obtained after 4 days of cultivation (Moreira *et al.,* 2007). Maximum yield of keratinase enzyme was obtained at 55°C. Keratinases production by the fungal strains *Trichoderma atroviridae* (Cao *et al*, 2008) *Doratomyces microsporus* (Gradisar *et al*, 2000), and *Aspergillus oryzae*, (Farag and Hassan, 2004) were seen at its maximum at similar optimum temperatures. In general, most of the keratinolytic proteases have the optimum temperatures between 50˚ C and 55˚C.

Feather of different particle size were tested for the production. Coarse powder was also tested as control. The enzyme production obtained was found to be low with the graded substrate in comparison with the coarse powder. However with particle size ranging from 500 -710 micron, a comparable yield was obtained.

The keratinase production was maximum at pH 9. Similar pH optima have been reported in the case of Doratomyces *microspores* (Gradisar *et al.,* 2000) and *Aspergillus fumigates* (Santos, 1996) and *Chrysosporium keratinophilum* (*Dozie et al.,* 1994). Different organisms have different pH optima and decrease or increase in pH on either side of the optimum value results in poor microbial growth. Among the tested carbon sources, all the organic carbon sources were found insignificant in keratinase production. Absence of carbon sources drove the fungus to assimilate the keratin as carbon source which increase the percentage of solubilisation, as the keratinolytic organisms are capable of using keratin as sole source of carbon and nitrogen. (Szabo *et al*, 2000). The work done by Gausterova*et al*, (2005), Malviya*et al.,* (1992) on *Chrysosporium queenslandicum* and El-Naghy*et al.,* (1998) on *Chrysosporium georgiae* noted that the keratinase enzyme was inducible by keratin substrate.

Table.1 Keratinase enzyme produced by top seven high yielding strains

Sl.No	Name of fungus	Keratinaseproduction (U/gds)
1	*Alternaria* S11	340.12
2	*A.flavus* S125	420.89
3	*A.fumigatus* S199	390.45
4	*A.nidulans* S241	300.01
5	*C.keratinophilum S252*	*350.23*
6	*Paeciliomyces* S341	270.26
7	*Doratomyces*S299	340.09
8	*C.keratinophilum* MTCC 1367	300.15

Table.2 Keratinase production by high yielding fungi in SSF system with different moisture levels incubated for different periods

Fungus	Substrate:MSS Ratio	Keratinase production at different incubation periods (U/gds)			
		3rd day	4th day	5th day	6th day
*Alternaria*S11	1:1	401.23	395.35	356.68	376.04
	1:1.5	403.54	398.24	331.30	345.09
	1:2	401.12	404.43	408.10	332.34
	1:2.5	325.56	368.78	343.95	339.60
*A.flavus*S125	1:1	433.12	446.23	421.25	403.45
	1:1.5	441.23	455.64	443.28	435.76
	1:2	426.20	432.25	486.90	450.13
	1:2.5	418.21	425.32	423.34	420.10
*A.fumigatus*S199	1:1	410.21	398.23	378.89	402.12
	1:1.5	346.21	367.56	366.45	356.45
	1:2	416.45	402.12	378.08	325.43
	1:2.5	356.23	354.23	345.78	298.56
A.nidulans S241	1:1	286.59	298.58	245.56	176.80
	1:1.5	254.28	288.56	245.67	201.34
	1:2	310.23	324.01	315.23	235.76
	1:2.5	267.34	278.89	298.26	212.34
C.keratinophilum S252	1:1	387.54	386.34	345.56	312.28
	1:1.5	353.12	368.32	298.67	297.45
	1:2	314.56	383.04	354.67	370.00
	1:2.5	315.34	321.10	323.23	328.34
Doratomyces S299	1:1	234.56	278.76	239.65	212.31
	1:1.5	258.89	254.67	214.34	189.45
	1:2	298.89	308.01	278.79	211.36
	1:2.5	234.65	276.56	213.45	201.13
Paeciliomyces S341	1:1	223.00	212.34	242.14	198.34
	1:1.5	243.32	234.45	225.34	202.34
	1:2	245.23	296.98	271.23	243.32
	1:2.5	212.12	232.12	201.21	186.34
C.keratinophilum MTCC 1367	1:1	298.45	261.12	221.40	189.41
	1:1.5	312.14	301.34	274.23	231.23
	1:2	332.56	324.45	297.10	243.30
	1:2.5	268.12	332.12	245.24	165.46

Table.3 Effect of different solid substrates on enzyme production

Solid substrates	Keratinase activity (U/gds)
Rice bran	177.58± 7.4
Wheat bran	426.49± 23.88
Wheat powder	223.39± 12.53
Coconut oil cake	35.03 ± 3.61
Ground nut oil cake	32.67 ± 2.45
Feather keratin powder	434.94 ± 32.18

Table.4 Effect of different particle size on keratinase production

Particle Size (Micron)	Enzyme activity U/ gds±SD
Ungraded	461.59±15.21
□ 100	170.36±10.28
100-250	240.50±32.26
250-500	294.42±12.30
500-710	389.52±25.27
710-1400	302.76±28.81

Table.5 Effect of Moisture content, temperature of incubation & incubation period

Ratio of substrate and moistening solution	Incubation Temperature	Keratinase production (U/gds) after different incubation periods			
		3 days	4 days	5 days	6days
1:0.5 (51.12%)	35	238±12.7	230±12.7	286±11.2	235±20.3
	45	248±10.9	232±10.9	278±19.0	246±14.1
	55	230±28.2	228±18.2	246±13.5	230±15.7
1:1 (55.03%)	35	343±13.2	345±13.2	263±15.3	241±9.1
	45	348±19.3	350±19.3	232±11.2	245±14.5
	55	342±12.2	343±22.2	298±12.1	238±13.1
1:1.5 (63.75%)	35	458±19.5	462±19.5	299±22.2	252±12.8
	45	462±23.2	468±23.2	390±27.2	360±18.1
	55	463±31.6	456±31.6	435±30.2	454±19.5
1:2 (69.92%)	35	572±30.8	578±30.8	482±21.1	462±20.3
	45	588±25.2	565±25.2	520±29.5	568±29.2
	55	621±31.1	632±31.1	625±33.6	609±30.2
1:2.5 (73.81%)	35	352±21.8	353±31.9	314±22.9	249±23.3
	45	355±23.1	358±20.1	254±18.3	250±21.6
	55	352±29.2	355±29.2	286±20.8	243±20.4

Table.6 Effect of various carbon sources on the enzyme production

Carbon source	Keratinase production u/gds ± SD
Coconut Oil	327.20±12.8
Dextrin	218.88±8.9
Sucrose	236.03±9.5
Starch	348.00±13.4
Glucose	344.20±16.5
Control	630.45±12.9

Table.7 Effect of organic nitrogen sources on enzyme production

Organic Nitrogen source	Keratinase production U/gds ± S.D
Peptone	76.40±6.79
Soy	247.77±20.12
Malt	128.25±8.12
Yeast	84.40±6.62
Casein	447.24±25.21
Urea	195.31±11.17
Control	651.03±20.21

Table.8 Effect of Inorganic Nitrogen sources on Enzyme production

Inorganic Nitrogen sources	Enzyme production U/gds±S.D
$CaNO_3$	399.84±13.07
NH_4NO_3	324.87±12.6
NH_4Cl	355.74±18.7
KNO_3	374.87±12.6
$NaNO_3$	358.93±15.89
Control	602.34±26.23

Table.9 Ratio of medium volume and flask volume

Media volume:Flask volume	Keratinase Production (U/gds)
1:14.8	500.53±24.3
1:7.8	620.25±30.2
1:4.5	640.45±35.54
1:3.8	540.32±28.34
1:3.2	467.70± 21.12
1:2.6	442.44± 23.23

Fig.1 Effect of pH of the moistening solution on the production of keratinase

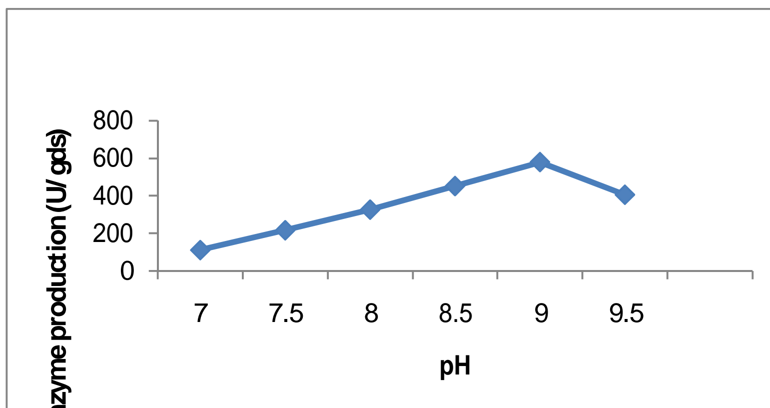


Fig.2 Keratinase production during feather degradation

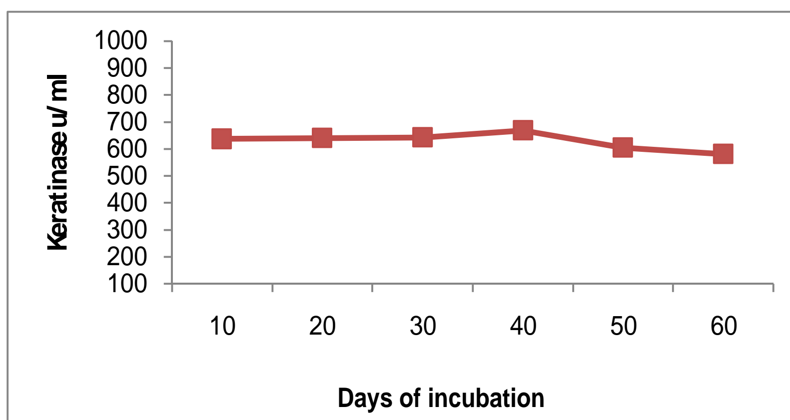
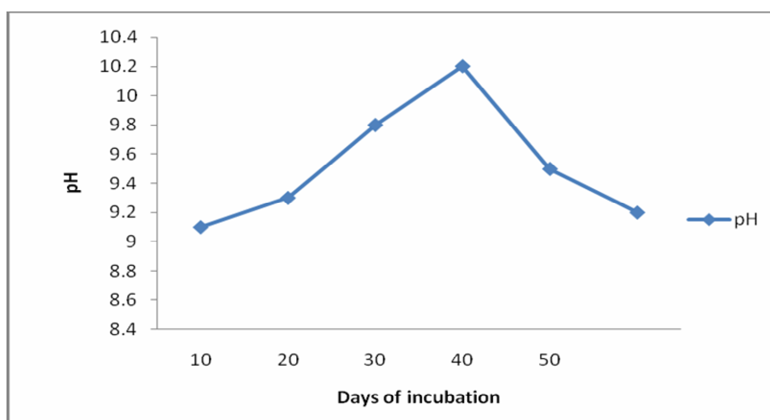


Fig.3 Change in pH of culture filtrate as a result of feather degradation by *A.flavus*



This study reports an inexpensive method for the keratinase production by utilizing chicken feather as substrate which in turn reduces the environmental pollution. By optimization the yield of keratinase from *A. flavus* S125 could be seen increased from 434.94 U/gds to 640.45U/gds. The main ingredient of the medium was feather and the feather alone could support the production of keratinase. So a detailed study on the degradation of feather by the fungus Aspergillus*flavus* S125 was conducted.

The activity of keratinolytic enzyme produced by *Aspergillus flavus*S125 effectively degraded feather substrate. In the feather degradation experiment pH was raising till the 40th day and there was a dip thereafter. Degradation of feather substrate was found to be associated with significant increase in pH of the medium to alkalinity, thus serving as an indicator for the efficiency of degradation.

The tendency of the medium to turn alkaline has been attributed to deamination reactions leading to production of ammonia from protein, amino acids and peptides during keratin degradation. Increase in alkalinity has also been observed to favour further rapid enzymatic attack of keratin resulting in higher levels of keratinolysis. (Riffel, 2003). It has been proposed that the high rate of keratinolysis resulted in the increased alkalinity of the spent medium. (Kumar &Kushwaha, 2014).

The high mechanical stability of keratin and its resistance to proteolytic degradation are due to the tight packing of the protein chains through intensive inter linkage by cystine bridges.

The capability of filamentous fungi to degrade keratin may be the result of a combination of extracellular keratinase, mechanical keratinolysis (mycelial pressure and/or penetration of the keratinous substrate), sulphitolysis (reduction of disulphide bonds by sulphite excreted by mycelia) and proteolysis (Gupta R. and Ramnani, 2006). In this study the preparation of feather powder supported the growth of the fungus as a sole carbon and nitrogen source. This indicated the potential of the *A. flavus* S125 for utilizing keratin to produce keratinase. The feather treated with microbial keratinase is attracting wide attention with several applications in recent years.

Keratinase-treated feather is increasingly considered as a viable source of dietary protein in food and feed supplements, as the enzyme-treated end product retained high nutritive value.

Moreover, the mounting issues of environmental pollution due to the accumulation of feather wastes from poultry farms could be minimised by these keratinases due to their effectiveness in feather degradation. Thus the capacity of the *A. flavus*S125 to produce keratinase by utilizing feather keratin substrate indicates the possible multiple benefits.

Although microorganisms have a potential to produce enzymes, production cost of the enzyme is the critical issue for further application at industrial level.

The degradation of poultry feathers by keratinolytic proteases offers an alternative method for efficient bioconversion, nutritional enhancement and environmental friendliness (Xu*et al*, 2009; AmareGessesse, 1997; Kumar *et al.,* 2004 and Enshasy*et al.,* 2008). In addition, the removal of these non-degradable wastes could minimize environmental pollution.

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