INTERNATIONAL JOURNAL OF SCIENCE AND NATURE

www.scienceandnature.org

EFFECT OF TEMPERATURE AND pH ON BACTERIAL DEGRADATION OF CHICKEN FEATHER WASTE (CFW)

[1]Onuoha, S.C. and [2]Chukwura, E.I.
[*1] Department of Science Laboratory Technology, Akanu Ibiam Federal Polytechnic Unwana, P.M.B, 1007, Afikpo, Ebonyi State, Nigeria, [2]Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, Awka , Anambra State

ABSTRACT
This study investigated the possibility of degrading chicken feathers discarded as environmental waste from poultry industry for the production of protein feedstuff for animal nutrition. Fourteen (14) bacterial isolates from chicken feather-waste were screened for feather degradation by growing them with feathers as their primary source of carbon, nitrogen and energy. One of the isolates designed as D_4 was characterized and selected for further studies based on its superior keratinolytic activity. The time course of study on bacterial growth and feather hydrolysis in liquid culture medium having raw feather as sole source of carbon and nitrogen was followed by measuring the release of free amino nitrogen (FAN), total non-protein nitrogen (TNPN) and medium optical density. Optimum growth and feather hydrolysis as well as maximum amino acid yield was achieved at thermophilic temperature of 50 and 60^0C. pH of 10.0 gave the optimum growth, yield of TNPN and FAN in the fermentation medium. These tests indicated that chicken feathers can be used in the local production of amino acid concentrate based on the isolate and also improve feather-based protein feeds for animal nutrition.

KEYWORDS: Feather, keratin, Hydrolysis, Protein, Fermentation, Bacillus, Enzymes

INTRODUCTION

Feathers which are produced in large amount as a waste globally have low commercial value. The disposal of such waste, often result in additional cost to the producers and their accumulation can lead to environmental problems. Such factors are resulting in growing interest in the utilization of agro-industrial waste in biotechnological processes. A current value-added use for feathers is the conversion to feather meal, a digestible dietary protein for animal feed, using physical and chemical treatments, other uses are as low cost substrates for the production of the enzymes and other value-added microbial products (Ko *et al*., 2010, Lateef *et al*., 2010, De Siqueira *et al*., 2010).

Bird feathers generally accumulate proteins, mainly keratin, which is the major class of structural proteins that are highly resistant to biological degradation (Ichida *et al*, 2001; Riffel *et al*., 2003; Ramnani *et al*., 2005). The keratins are insoluble, and are not easily degradable by common proteolytic enzymes while in its native form (Gousterova *et al*, 2005). The mechanical stability and resistance to proteolytic digestion are consequences of the high degree of cross-linking of the polypeptide chain caused by extensive formation of disulphide bonds and hydrogen bonding among the polypeptide. Hydrolyzing the feather into cysteine-rich high product produces feather meal. It involves hydrothermal degradation whereby feathers are steam pressure-cooked, a method similar to autoclaving by drying.

But producing feather-meal through the above process requires significant energy and destroys certain amino acids; it also causes the formation of non-nutritive amino acids such as lysino-alanine and lanthionine (Wang and Parson, 1997). In spite of its limitations, this meal is incorporated into diet on as-fed in chicken poultry (40-50g/kg), rainbow trout (150g/kg), shrimp (350g/kg) and salmon (400g/kg), but the resulting diet needs amino acid supplementation, especially feed-grade lysine and others (Cheng *et al*, 2002., Brandsen *et al*, 2001)

In view of the thermo energetic cost of the conventional processing of feather against the backdrop of its limited nutritional improvement, investigations into alternative technology with prospects for nutritional enhancement, environmental compatibility, bioresource optimization seems justifiable (Onifade *et al*, 1998). Both the digestibility and amino acid balance of feather meal might improve by microbial action. . A number of keratinolytic microorganisms have been reported, including some species of Bacillus and Streptomyces (Kim *et al*, 2001; Daroit *et al*.; 2009, 2011), some gram positive bacteria such as Arthrobacter sp (Lucas *et al*, 2003), Microbacterium sp (Thys *et al*, 2004 and Kocuria rosea (Bernal *et al*, 2006) and gram-negative bacteria such as Vibrio sp (Sangali and Brandalli, 2000), Xanthomonas maltophila (De Toni *et al*, 2002), Chryseobacterium sp (Riffel *et al*, 2003; Wang *et al*, 2008).

Screening for non- pathogenic microorganism with keratinolytic activity may prevent the need for isolation and purification of the enzymes. Their biomass could autolytically contribute to the protein and amino acid content of the fermented feathermeal.The upgrading of the nutritional value of the feathers should yield an enhanced protein feedstuff that may reduce the use of soybean and fish meal in livestock diets.

The objectives of this study is to determine the effect of some environmental factors on the bacterial degradation of chicken feather wastes in order to harness the possibility

of producing feather-based protein feed- stuff for animal nutrition.

MATERIALS AND METHODS

Collection of sample and isolation of microorganism:

Feathers were collected from several sites within a local poultry farm in Abakaliki, Ebonyi State, Nigeria in September 2009 and the work conducted between September and December 2009. The feathers were incubated in $5gl^{-1}$ peptone solution at 30^oC for 24 h. Bacterial suspensions were streaked on feather meal agar plates ($15gl^{-1}$ feather meal, $0.5gl^{-1}$ NaCl, $0.3gl^{-1}$ K_2HPO_4, $0.4gl^{-1}$ KH_2PO_4 and $15gl^{-1}$ agar) which were incubated at 37^oC for 1-5d. Single colonies were isolated and screened for their ability to hydrolyze keratin in feather meal agar plates.

Collection and Procession of Chicken Feathers

Fresh chicken feathers were obtained from chicken rendering house in Abakaliki, Ebonyi State, Nigeria. Strong and healthy ones (quill feathers) were selected in the laboratory and washed very well with sterile distilled-water. Washed feathers were dried at 50^oC in a forced draught oven (Gallenkamp, Ltd UK). The dried feathers were ground into fine fractions (<90, 90, 150, 300, 425 and 850µM) with test sieves of appropriate diameters.

Screening for Feather Degradation

Degradation of feathers by the isolates was demonstrated in large test tubes (20 x2.5cm) using the method of Williams *et al*, (1990). Each test tube containing 29mL mineral salt medium (MSM), 2g of dried native feather and plugged with cotton wool was sterilized at 121^oC for 15min. The tubes with their contents were each inoculated with equal amounts of washed cells of the bacterial cultures. Inoculated controls were incubated at 50^oC in the water bath for 7 days under static-conditions.

Effect of Temperature on the Growth and Hydrolysis of Feather by Bacterial Isolates.

Medium and conditions for this study were as above except that only three of the isolates (viz: A_1, A_5 and D_4) were used. An aliquot (0.3-0.5 ml) of the inoculums suspension of the cells representing 2x10 cfu/ml was used to inoculate 18, 250 ml Erlenmeyer flask, each with 50 ml sterile MSM medium. Inoculated flasks were incubated at 50, 55, and 60^oC under static condition in a water bath for 7 days. Rates of feather degradation by the isolates were monitored daily by withdrawing about 5 ml samples and assaying for free amino acid levels.

Effect of Medium pH on the Growth of Isolate D_4 and Hydrolysis of Chicken Feather

MSM medium (50ml) was dispensed into each of 10 duplicate 250ml Erlenmeyer flasks. The pH of the duplicate flasks were adjusted to 6.0, 7.0, 8.0, 9.0 and 10.0 with 0.1N HCl and 0.1n NaOH, using a pye Unicam pH meter (model 90MK2, Cambridge, UK). Then, 2.5g of ground feather (particle size 300µM) were added to each flask before sterilization at 121^oC for 10 min. Another set of 10 duplicate, 250ml Erlenmeyer flask was prepared as above. All the flasks were inoculated with saline suspension of isolate D4. One set was incubated in water bath shaker, while the other set was incubated under static condition in kottermann water bath. Samples were collected on daily basis and analyzed for free amino group, total non-protein nitrogen and biomass change.

Statistical analysis:

The results obtained were expressed as mean ± standard deviation (SD) for triplicate determination. Data were analyzed using paired `T'. Difference between means were significant at $P<0.05$

RESULTS

Screening of Isolates for Feather Degradation

The result of the screening test of the isolates on feathers (Table 1) show that all the fourteen (14) bacterial isolates recovered from the samples were able to grow on the whole chicken feather as its primary source of carbon, nitrogen and energy, but the degree of degradation varied with each isolate after 7 days of incubation at room temperature (25^oC). The isolates were designated as A_1, A_2, **A_3, A_4, A_5, A_6, K_1, K_2, K_3, and K_4, D_1, D_2, D_3 and D_4**. Isolates- **A_4, K_3, K_4, D_1** and D_2 degraded feather vanes but not feather shaft, while, isolate **A_2, A_3, A_6, K_1**, and **K_2** showed extensive degradation of vanes plus slight degradation of shaft. Only three (3) isolates**, A_1, A_5** and **D_4** showed extensive degradation of both feather vanes and shafts. The culture of isolate D_4 displayed some breakdown of the feather quill as well.

TABLE 1. Feather degradation by isolates

S/N	Isolate designation	Extents of feather degradation
1.	A_1	+ + + +
2.	A_2	+ + +
3.	A_3	+ + +
4.	A_4	+ +
5.	A_5	+ + + +
6.	A_6	+ + +
7.	K_1	+ + +
8.	K_2	++ +
9.	K_3	++
10.	K_4	++
11.	D_1	++
12.	D_2	++
13.	D_3	+++
14.	D_4	++++

++ = Degradation of feather vanes

+++ = Extensive degradation of feather vanes plus slight degradation of feather shaft.

++++ = Extensive degradation of feather vanes and shaft.

Characterization of the Isolates

The isolate D_4 which showed the most degradation of feather vanes, shafts and quill was characterized. The result of the characterization showed that isolate D_4 was a gram positive, spore-forming straight rods occurring singly or in pairs and were motile. It was also catalase positive; hydrolyzed starch, showed a negative reaction in arginine test, positive reaction in nitrate reduction and voges-proskaur test. It also produced acid from mannitol fermentation. It grew well on nutrient agar medium incorporated with 7% sodium chloride (NaCl) salt. The isolate was therefore identified as a *Bacillus* species based on the outline in Bergey's manual of systematic bacteriology (Kreig and Holt, 1986).

Effect of Temperature on Growth and Chicken feather Degradation by isolate A_1, A_5 and D_4

Isolates A_1, A_5 and D_4 degraded feathers at 50, 55 and 60^0C respectively. Percentage feather breakdown for A_1, A_5 and D_4 were 20, 22.7 and 20 % (w/w) respectively at 50^0C, but 20, 40 and 40 %(w/w) at 55^0C and at 60^0C the amount of feather hydrolyzed by the isolates increased to 25 and 44 % respectively for isolates A_1 and D_4, but remained 40% for A_5, percentage feather breakdown was highest at 60^0C growth temperature for all the isolates. A comparison of the level of feather degradation by the isolates (Fig1) showed that at 50^0C A_1 was less than D_4, while D_4 was less than A_5. At 55^0C, A_1 was less A_5, which was equal to D_4, at 60^0C A_1 was less than A_5, while A_5 was less than D_4 (Fig1).

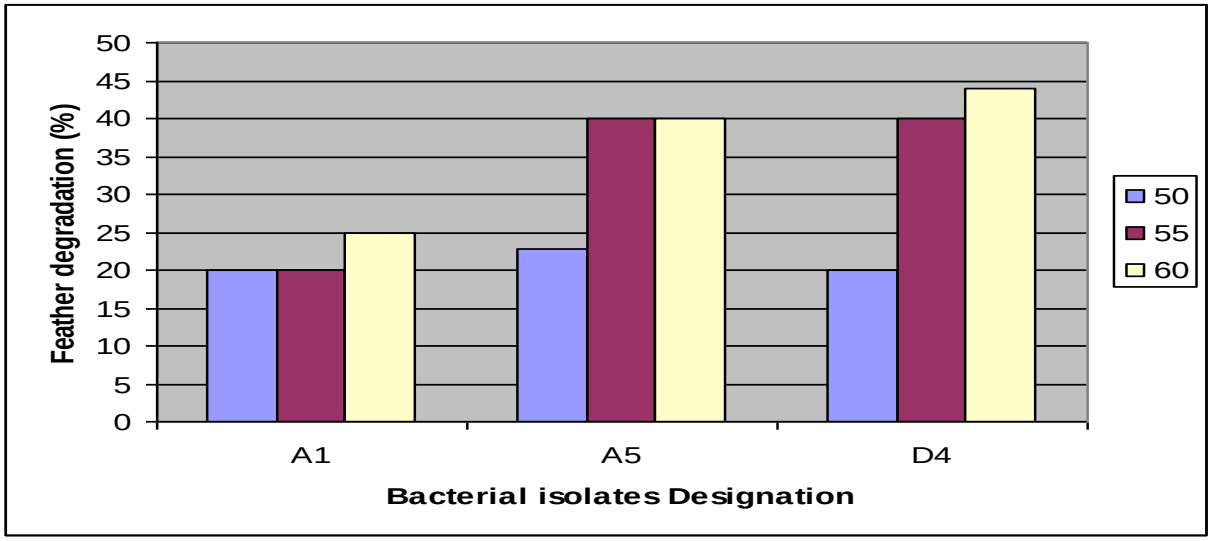

FIGURE 1. Effect of Temperature on Feather Degradation by Bacterial Isolates A1, A5, D4

Effect of temperature on amino acid production by isolates A_1, A_5 and D_4

The temperature of growth and bacterial isolates influenced total amino nitrogen in culture filtrate. Generally, amino nitrogen level constantly increased during the period of fermentation. Peak amino levels for the isolates were as follows at 50, 55 and 60^0C respectively, isolate A_1 1.88, 2.35 and 2.00, isolate A_5 1.80, 4.55 and 4.60 and isolate D_4 1.58, 3.80 and 6.00 mg/l. maximum amino acid level from chicken feather (60 mg/l) was achieved with isolate D_4 at 60^0C incubation temperature, isolate A_1 and A_5 produced maximum amino acid (2.35 and 4.60 mg/l) at 55 and 60^0C respectively. From figure 2 ,3 and 4 , it could be observed that isolate A_1 irrespective of temperature of incubation gave the least amino nitrogen level. Based on the above result and those of Figure 1 isolate D_4 was selected for further studies

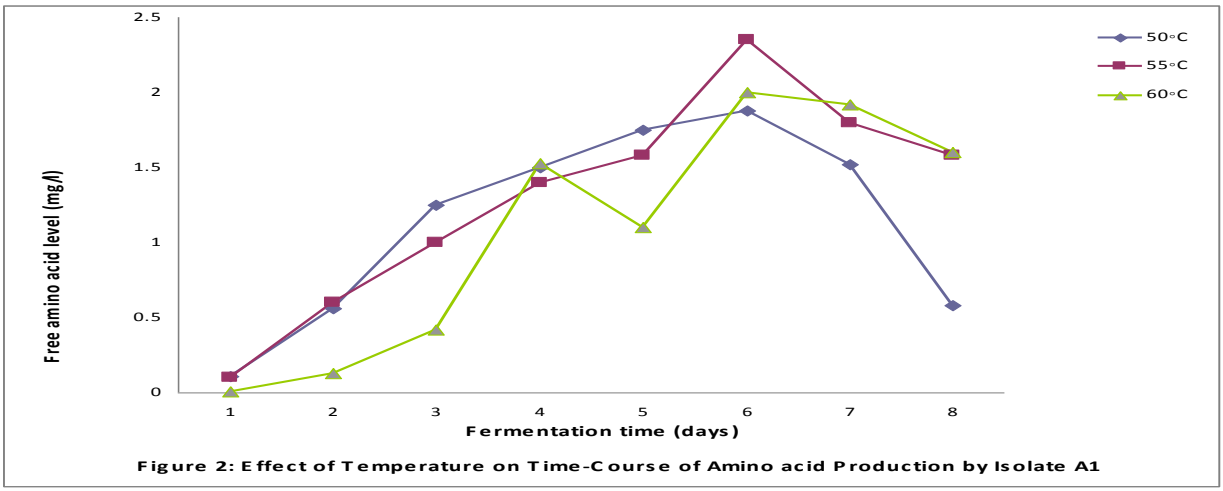

Figure 2: Effect of Temperature on Time-Course of Amino acid Production by Isolate A1

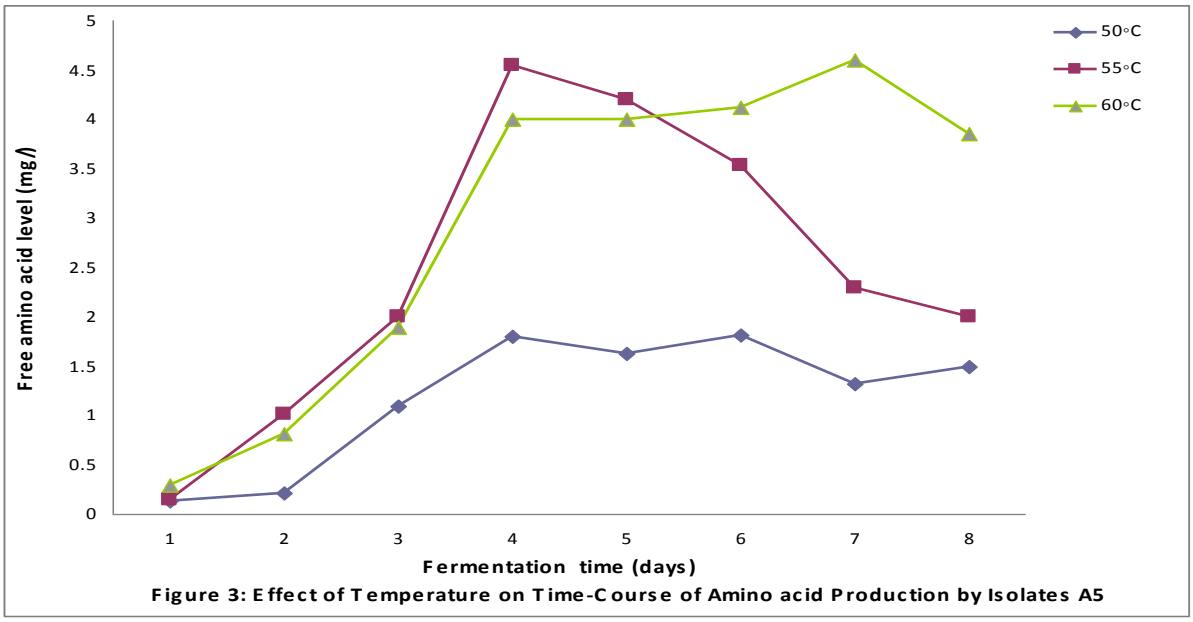

Figure 3: Effect of Temperature on Time-Course of Amino acid Production by Isolates A5

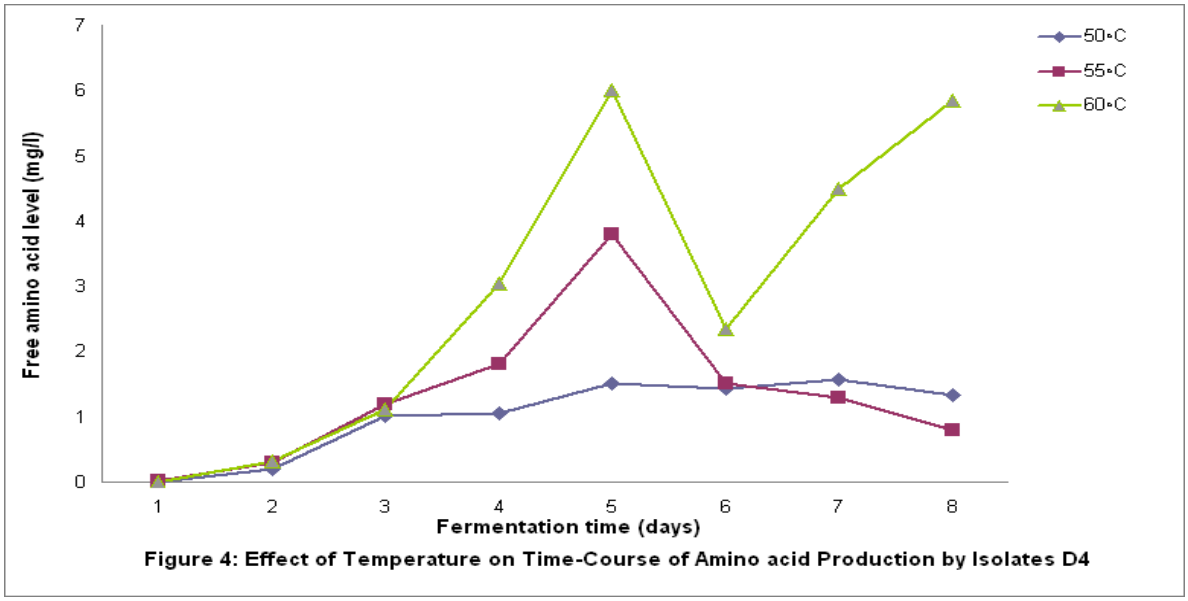

Figure 4: Effect of Temperature on Time-Course of Amino acid Production by Isolates D4

Effect of initial medium pH

Maximum growth or biomass yield is shown in table 2. Initial medium pH influenced growth of isolate D_4 on feather. Rates of microbial growth rose as initial medium pH increased from pH 6.0 upwards reaching peak at pH 10.00 for both culture. Shake flask cultures gave more biomass than static cultures irrespective of pH. Statistically, there was no significance difference in the mean of both cultures

TABLE 2: Effect of medium pH and cultivation mode on Isolate D_4 Growth

Medium pH	Maximum growth ($0D_{600nm}$) in Static-culture	Shake-flask culture
6.0	2.40± 0.01	2.80± 0.02
7.0	2.30± 0.11	2.40± 0.03
8.0	2.50 ± 0.01	3.30 ± 0.11
9.0	2.00± 0.02	2.30 ± 0.10
10.0	3.50 ± 0.03	.00 ± 0.02

Results represent the mean ± standard deviation of triplicate determination

Effect of initial medium pH on amino acid production

Maximum amino acid production for shake flask and static cultures is shown in table 3. Maximum amino acid production was attained at pH 10.0 (38.1mg/l) for shake flask, while that of static cultures was attained at pH 8.0 (40.0mg/l). In general, rate of release of amino acid were higher in shake flask grown cell than in static cultures, but the increase was not significant (P>0.05) in the release of amino acid in both cultures

TABLE 3: Effect of medium pH on free amino acid release by Isolate D_4

Free amino acid (mg/l) in Medium pH	Static-culture	Shake-flask culture
6.0	14.70± 0.02	31.70± 0.02
7.0	30.00± 0.03	13.30± 0.02
8.0	40.00 ± 0.11	35.00 ± 0.04
9.0	22.50± 0.03	15.00 ± 0.10
10.0	30.00 ± 0.01	38.10 ± 0.02

Results represent the mean ± standard deviation of triplicate determination

TABLE 4: Effect of medium pH on total non-protein nitrogen release by Isolate D_4

Maximum total non-protein nitrogen ($x10^2$ mg/l) Medium pH	Static-culture	flaskculture
6.0	2.30± 0.03	2.56± 0.02
7.0	1.80± 0.02	1.00± 0.03
8.0	1.54 ± 0.02	2.08 ± 0.02
9.0	1.61± 0.03	1.63± 0.01
10.0	2.00 ± 0.11	3.20 ± 0.01

Results represent the mean ± standard deviation of triplicate determination

Effect of Initial Medium pH on Total Non-Protein Nitrogen Production (NPN)

The effects of medium pH on the rate of non-protein nitrogen release by isolate D_4 is presented in table 4. Then maximum NPN production was achieved at pH 10.0 for shake-flask culture, while maximum NPN production was achieved at pH 6.0 for static culture. Statistically, the difference between the mean in both culture were insignificant

DISCUSSION

Bacteria were isolated from waste chicken feathers, they were found to be capable of hydrolyzing feathers. The isolate grew optimally and hydrolyze chicken feather at a thermophilic temperature of 50 and 60^0C respectively.

Preliminary identification test indicate that isolate D_4 was a *Bacillus* species. Corroborating these findings Atalo & Gashe, (1993) and Kim *et al*, (2001) previously isolated keratinolytic bacteria at elevated temperatures. Also, the most studied keratinolytic bacteria are *Bacillus* sp, which have been described to possess feather-degrading activity (Kim *et al*, 2001; Lin *et al*, 1999). The isolate grew optimally and hydrolyzed chicken feather at a thermophilic temperature of 50^0C.

The bacterial isolate used in this study is essentially aerobic. Aerobic growth of the isolate on chicken feather yielded appreciable levels of degradation of the untreated feather substrate as measured by the release of free amino acids in the fermentation broth as well as by the visual observation of the physical-disintegration of the feather substrate. Williams *et al*, (1990) observed in his study a similar heavier growth of *Bacillus licheniformis* PWD-1 under aerobic conditions.

It is possible that better aeration under agitated culture condition could in part be responsible for the better microbial growth observed throughout this work under shake flask condition. Amino acid liberation was slower and lower under static conditions, also higher level of TNPN were recorded for agitated cultures, but the result was not statistically significant P>0.05. This result however agrees with the work of Williams *et al*, (1990) and Takami *et al* (1992) who observed similar results when they concluded that growth in agitated cultures because of higher substrate: enzyme contact rates encouraged a rapid catabolism of newly released non-free amino acid and non-protein nitrogen.

The resistance of keratin to proteolytic digestion has been associated with their super-coiled helical structure (Lin *et al*., 1992; Onifade *et al*., 1998 and Lin *et al*., 1996). Ball milling is one of the methods that is employed in the conversion of keratin containing substances such as chicken feather to a more degradable product. The milling process modify the recalcitrant native keratinous structure of feather to a form that is readily attacked and degraded by enzymes which might not be specific for chicken feather e.g. trypsin. Since the milling of the feather substrates involves the degradation of its complex structure, it is assumed that the ever more rigorous mechanical process involved in the preparation of increasingly finer feather substrates types would have resulted in progressively more extensive keratinous structure modification

The pH of microbiological growth media is one of the most important factors that modulate microbial fermentative productivity (El-Shora *et al*., 1993). The growth on feather by isolate D_4 was studied in relation to the pH of the liquid fermentation medium.

The growth velocities of the isolate were higher at increasingly more alkaline medium pH except for pH 7.0 and 9.0. The fall in rates of microbial growth at the two pH could be due to lowered enzyme stability under these conditions. This view is supported by data on enzyme pH stability which reveal that enzyme grew increasingly unstable as pH become more alkaline and may also explain the lower free amino acid and TNPN levels recorded at that ranges of pH. Growth under static culture condition was also optimal at pH of 10.0 also; amino acid release was also influenced by the pH of the fermentation

medium. But there was no significant difference P>0.05 in both culture conditions tested. High rate of amino acid and TNPN release observed at pH of 10.0 may have been the results of improved feather substrate digestibility emanating from exposure to the alkaline condition of the fermentation medium. To further corroborate our findings an increase in pH values was equally observed during feather degradation according to studies conducted by Kaul *et al*, (1997) and Sangali & Brandelli, (2000). This trend may be associated with proteolytic activity, consequent deamination reactions and the release of excess nitrogen and ammonium ions. The increase in pH during cultivation is pointed as an important indication of keratinolytic potential of microorganisms (Kaul *et al*, 1997).

CONCLUSION

The results revealed that a thermophilic bacterial isolate from chicken feather waste was used in the degradation of feather keratin using feathers as a primary source of energy and carbon. The isolate was classified as *Bacillus* species.

When the isolate was grown aerobically on feather medium, it yielded appreciable degradation products measured by the release of free amino acids and TNPN in the fermentation medium. The released free amino acids are essential to animal nutrition, suggesting the possible use of biotechnological process based on the isolate in local production of animal nutrition.

REFERENCES

Atalo, K. and Gashe B.A. (1993) Protease production by a thermophilic *Bacillus* species (P-001A) which degrade various kinds of fibrous proteins. *Biotechnol. Lett*., 15: 1151-1156.

Baron, J.E., Bailey, W.R., and Finegold, S.M. (1990) Methods for Testing Antimicrobial Effectiveness. In: Bailey and Scott's Diagnostic Microbiology, Baron, E.J. and S.M. Finegold (Eds.). 8th Edn. Mosby, Missouri, USA., ISBN-13: 9780801603440, pp: 861

Brandsen, M., Carter, C. and Nowak, B. (2001) Effect of dietary protein sources on growth, immune function, blood chemistry and disease of Atlantic Salmon(*Salmo solar L) Parr*. *Animal Science*, 73, 105-113.

Bernal, C., Diaz, I., and Coello, N. (2006) Response surface methology for the optimization of keratinase production in culture medium containing feathers produced by *Kocuria rosea*. *Can. J. Microbiol*, 52, 445-450

Cheng, Z., Behnke, K., Dominy, W. (2002) Effect of feather meal on growth and body composition of the juvenile pacific white shrimp *Litopenaeus vannamel*. Journal Appl. Aquaculture, 12(1) 57-69.

Daroit, D.J., Correa, A.P.F. and Brandelli, A. (2011) Production of keratinolytic proteases through bioconversion of feather meal by the Amazonian Bacterium *Bacillus species* P45. *Int. Biodeterior. Biodegrad*, 65: 45-51.

De Siqueira, F.G; de Siqueira, E.G.; Jaramilo, P.M.D., Silveira, M.H.L. and Andreaus, J. 2010. The potential of agro-industrial residues for the production of holocellulase from filamentous fungi. Int. Biodetrior. Biodegrad., 64: 20-25.

De Toni, C.H., Andreaus, M.F.C., Chagas, J.R. , Henriques, J.A and Termignoni, C. (2002) Purification and characterization of an alkaline serine endopeptidase from a feather-degrading *Xanthomonas maltophila* strain. Can J. Microbiol, 48, 342-348

Daroit, D.J., Correa, A.P.F. and Brandelli, A. (2009) Keratinolytic potential of a novel *Bacillus* sp. P45 isolated from the Amazon basin fish <I>Piaractus mesepotanicus. *Int. Biodeterior. Biodegrad*, 65: 358-363.

El-Shora, H.M., Ashour, S.A. and Ghanem, A.A., (1993) Purification and Characterization of keratinase from Bacillus circulans, B. licheniformis & B. megaterium. *J. of Environ. Science Mansoura Univ. Egypt*, 6: 89-103.

Gousterova, A. ., Braikova, D., Goshev, I., Chritov, P., Tishnov, K., Tonkova, V.E ., Haertle, T., Nedkov, P. (2005) Degradation of keratin and collagen containing wastes by newly isolated *Thermoactinomycetes* or by alkaline hyrolysis.Letters in Appl. Microbiol. 40, 335-340.

Ichida, J.M., Krizova, L., LeFevre, C.A., Keener, H.M., Elwell, D.L. and Burtt Jr E.H. (2001) Bacterial inoculums enhance keratin degradation and biofilm formation in poultry compost. *J. Microbiol. Methods*, 47: 119-208.

Kaul, S., and Sambali, G. (1997) Keratinolysis by poultry farm soil fungi. Mycopathologia, 139, 137-140

Ko, C.H., Lin, Z.P., Tu, J., Tsai, C.H., Liu, C.C., Chen, H.T and Wang, T.P. (2010) Xylanase production by *Paenibacillus campinasensis* BL11 and its pretreatment of hardwood kraft pulp bleaching. *Int. Biodeterior. Biodegrad*, 64: 13-19.

Kreig, N.R. and Holt, J.G.W. (1986) Bergy's Manual of Systematic Bacteriology. Vol. 2 and 3. *Williams and Wilkins, Baltimore,* USA.

Kim, J.M., Lim, W.J. and Suh, H.J. (2001) Feather-degrading *Bacillus* species from poultry waste. Process *Biochem*, 37, 287-291

Lee, C.G.M, Ferket, P.R. and Shih, J.C.H. (1991) Improvement of feather digestibility by Bacterial keratinase as feed additive. *FASEB J*., 59: 1312-1312.

Lateef, A., Oloke, J.K., Kana, E.B.G., Shobowale, B.O., Ajao, S.O. and Bello, B.Y. (2010) Keratinolytic activities of a new feather-degrading isolate of *Bacillus cereus* LAU08 isolated from Nigerian soil. Int. *Biodeterior. Biodegrad*., 64:162-165.

Lin, X., Lee, C.G., Casale, E.S and Shih, J.C. (1992) Purification and characterization of a keratinase from a

feather degrading *Bacillus licheniformis* strain. *Applied Environ.Microbiol*., 8: 3271-3275.

Lin, X., Shih, J. and Swaisgood, H.E. (1996) Hydrolysis of feather keratin by immobilized keratinase. *Applied Environ. Microbiol*., 62: 4273-4275.

Lucas, F.S., Broennimann, O., Febbraro, I and Heeb, P. (2003) High diversity among feather-degrading bacteria from a dry meadow soil. *Microb. Ecol*., 45, 282-290

Onifade, A.A., Al-Sane, N.A., Al-Musallam, A.A. and Al-Zaratan, S. (1998) A review: Potential for biotechnological applications of keratin-degrading microorganisms and their enzyme for nutritional improvement of feathers and other keratins of livestock feed resource. *Bioresour. Technol*., 66: 1-11.

Ramnani, P., Singh, R. and Gupta, R. (2005) Keratinolytic potential of *B. licheniformis RG1*: Structural and biochemical mechanism of feather degradation. *Can. J. Microbiol*., 51: 191-196.

Riffel, A., Lucas, F., Heeb, P. and Brandelli, A. (2003) Characterization of a new keratinolytic bacterium that completely degrades native feather keratin. *Arch. Microbiol*., 179: 258-265.

Sangali, S and Brandalli, A. (2000) Feather keratin hyrolysis by a *vibrio* sp.strain kr2. *Journal Appl Microbiol*, 89(5), 735-743.

Takami, H., Nakamura, F., Anon, R and Horishiri, K. (1992) Degradation of human hair by a thermostable alkaline proteinase from alkalophilic *Bacillus sp*. No. AH 101. *Biosci. Biotechnol. Biochem*., 56: 1667-1669.

Thys, R.C.S., Lucas F.S., Riffel,A., Heeb, P and Brandelli, A. 2004. Characterization of a protease of a feather-degrading *Microbacterium* species. Letters in *Appl. Microbiol*., 39, 181-186.

Vidali, L., Christen, P and Coello, N. (2000) Feather degradation by *kocuria rosea* in submerged culture. *World Journal Microbiol. Biotech*. 16, 551-554.

Wang, X. and Parsons, C.M. (1997) Effect of processing systems on protein quality of feather meal and hair meals. *Poult. Sci.*, 76: 491-496.

Wang, S.L., Hsu, W.T., Liang, T.W., Yen, Y.H and Wang, C.L. (2008) Purification and characterization of three novel *keratinolytic metalloproteases* produced by *Chryseobacterium indologenes* TKU014 in a shrimp shell powder medium. Bioresource technology, 99, 5675686.

Williams, C.M., Richester, C.S., Mackenzie, J.M and Shih, J.C. (1990) Isolation, Identification and Characterization of a feather degrading bacterium. *Applied Environ. Microbiol*., 56: 1509-1515

.

Williams, C.M., Lee, C.G., Garlich, J.D and Shih, J.C.H. (1990) Evaluation of a bacterial feather fermentation product, feather lysates a feed protein. *Poult. Sci*., 70:85-94