Acknowledgement

I express my heartfelt gratitude to my mentor, Dr. T. VIRUTHAGIRI, Professor of Chemical Engineering, Department of Chemical Engineering, Annamalai University, for his inspiring guidance, for suggesting the topic, tremendous support and encouragement throughout the course of my doctoral programme. He encouraged me from time to time and offered moral support during the course of my research work. I consider it is a rare opportunity and great honors to work under him.

I am very much thankful to the most respected Vice Chancellor, Dr. S. Manian, Respected Registrar, Dr. K. Arumugam and distinguished Dean, Faculty of Engineering and Technology, Dr. C. Antony Jeyasehar and beloved Head of the Department, Department of Chemical Engineering, Dr. P. Kantha Bhabha for providing the faculties to carry out my research work.

I am bound to acknowledge Dr. K. Vimalashanmugam Assistant professor in Chemical Engineering for his much needed help throughout my research work, and for having enthused me when the work was tough going, Dr. T. R. Manikkandan Assistant Professor in Chemical Engineering, for his valuable suggestions and technical discussions in carrying out the experiments.

I thank B. Sivaprakash Assistant Professor in Chemical Engineering for his unstinted help in my experimental modeling work, for his much needed help and constant support, Dr. R. Rajesh Kannan Assistant Professor in Chemical Engineering and V. Selvi Assistant Professor in Chemical Engineering, for their suggestions and discussions in statistical design of experiments, Mr. K. Muthu Assistant Professor in Chemical Engineering for their constant support.

I also thank Mr. S. K. Mohan, Research Scholar, Department of Chemical Engineering for his timely help during the organization of the thesis.

I am very thankful to Mr. S. Manohar, Mr. S. Nagaiyan and Mrs. M. Sumathi, Instructor (Spl. Grade) and the other Technical Assistants Mr. T. Pasupathi, Mr. K. Ravikumar, in the Bioprocess Laboratory, Department of Chemical Engineering, Annamalai University for their help during the course of the research work.

I owe my heartfelt thanks to many others unnamed here, who offered their help for the successful execution of this work. Finally, I will be failing in my duty if I don’t put on
record the sacrifices and patience of my daughter K. Divyadharshini and my family members, during the entire course of my research work.

I thank all the professors and other faculty members of the Department for their constant encouragement and support.

I also thank all the non-teaching staff who helped me for carrying out my research particularly.

K.REVATHI
ABSTRACT

Microbial keratinase is one of the most important enzymes and finds lot of applications in the hydrolysis of highly rigid, strongly cross-linked structural polypeptides “Keratin” recalcitrant. Keratins are insoluble fibrous proteins found in hair, wool, feather, nail, horns and other epithelial covering contains beta helical coil which is linked through cysteine bridges. Keratinases are produced by several bacteria, fungi and actinomycetes isolated from terrestrial and marine sources. The bioconversion of insoluble feather keratin to soluble feather residue has high nutritional values and can be used as a supplement for livestock feeds. Other promising applications are in the field of keratin elimination in acne, depilation process, vaccine for dermatophytosis therapy, pharmaceutical enhancement of nail treatment and degradation of prion and prion - like proteins. Microbes serve as a preferred source for proteases are particularly derived from Bacillus strains.

The present research is aimed at maximum production of keratinase enzyme from bacterial strains namely Bacillus subtilis NCIM 2724 and Streptomyces griseus 2622 using chicken feather powder, goose feather powder, rice husk, sugarcane baggase, fish scales and shrimp shell powder as substrates. Among the six substrates chicken feather powder, goose feather powder and rice husk were found to produce maximum keratinase activity of 22.83 IU/ml, 16.32 IU/ml and 14.63 IU/ml respectively at an incubation time of 48 hours using B. subtilis. Similarly using S. griseus the maximum keratinase activity was found to be 17.12 IU/ml , 15.05 IU/ml and 12.38 IU/ml with chicken feather powder, goose feather powder and rice husk respectively with an incubation period of 72 hours. Since the substrates CFP, GFP and RH gave maximum keratinase activity and were selected for further optimization studies.

The effect of five important variables namely incubation period, temperature, initial pH, substrate concentration (Chicken feather powder) and inoculum size on keratinase enzyme production was studied with the
Feather Meal Media (FMM) using *B. subtilis* NCIM 2724 by classical method. The optimum conditions by classical method were found to be 48 h incubation period, 40\(^0\)C, initial pH 8.0, substrate concentration (1.5% w/v) and inoculum size (4% v/v) were based on maximum keratinase enzyme activity. Similarly the effect of five parameters have been studied using *S. griseus* NCIM 2622. The maximum keratinase production was found at 50\(^0\)C, (1.5% w/v) substrate concentration, initial pH 9, incubation period 72 h and inoculum size (4% v/v) respectively. Similar results were obtained for the remaining substrates goose feather powder and rice husk.

Plackett Burmann design was used to identify the most significant variables affecting the keratinase enzyme production using *Bacillus subtilis* NCIM 2724 with CFP as substrates. The effect of factors namely yeast extract, NH\(_4\)Cl, KH\(_2\)PO\(_4\), NaCl, KCl, MgSO\(_4\), FeSO\(_4\).7H\(_2\)O, Peptone, NaNO\(_3\), urea and K\(_2\)HPO\(_4\) on keratinase production was studied by conducting 12 experiments using chicken feather powder as substrate. From the results the most significant components affecting keratinase production were found to be peptone, NaCl, KCl and K\(_2\)HPO\(_4\) and were selected for further optimization studies using CCD and RSM. Similar experiments were carried out with goose feather and rice husk as substrates using *B. subtilis*. Studies on keratinase production with *streptomyces griseus* NCIM 2622 using P.B design was carried out in the same way with the three substrates and most significant variables were identified in each case.

Further optimization on media components was carried out using Central Composite Design (CCD) and Response Surface Methodology (RSM). The optimum values of the most significant factors obtained for maximum production of keratinase enzyme using *B. subtilis* with various substrates are given below:

(i) Chicken feather powder (CFP): Peptone -0.50 % w/v, NaCl - 0.110 % w/v, KCl - 0.110 % w/v and K\(_2\)HPO\(_4\) - 0.130 % w/v.

(ii) Goose feather powder (GFP): Urea-0.09999% w/v, yeast extract -0.49955% w/v, FeSO\(_4\).7H\(_2\)O -0.3997% w/v and NaNO\(_3\) -0.2% w/v.
(iii) Rice husk (RH): Urea - 0.1003\% w/v, yeast extract-0.50015 \% w/v, 
\(\text{NH}_4\text{Cl}-0.808 \% \text{w/v}\) and \(\text{FeSO}_4\ \text{7H}_2\text{O} -0.402 \% \text{w/v}\).

The optimum values of the most significant factors obtained for maximum production of keratinase enzyme using \textit{S. griseus} with various substrates are given below:

(i) Chicken feather powder (CFP): Peptone - 0.49985 \% w/v, \(\text{NaNO}_3\) -0.203 \% w/v, \(\text{FeSO}_4\ \text{7H}_2\text{O} -0.4055 \% \text{w/v}\) and \(\text{MgSO}_4\ 0.1001 \% \text{w/v}\).

(ii) Goose feather powder (GFP): \(\text{FeSO}_4\ \text{7H}_2\text{O} -0.399 \% \text{w/v}\), yeast extract -0.5015 \% w/v, \(\text{NaCl} -0.11015 \% \text{w/v}\) and \(\text{NH}_4\text{Cl} -0.804 \% \text{w/v}\).

(iii) Rice husk (RH): yeast extract -0.4985\% w/v, \(\text{K}_2\text{HPO}_4 -0.1308\% \text{w/v}\), \(\text{KCl} -0.1115\% \text{w/v}\) and \(\text{FeSO}_4\text{7H}_2\text{O} -0.404\% \text{w/v}\).

The optimum conditions of the most significant factors obtained for maximum production of keratinase enzyme using \textit{B.subtilis} with various substrates are given below:

(i) Chicken feather powder (CFP): Incubation period - 46.846 h, temperature- 41.681\(^{\circ}\text{C}\), pH - 8.1681, substrate concentration -1.5120 \% w/v and inoculum size - 3.9898 \% v/v.

(ii) Goose feather powder (GFP): Incubation period- 48 h, temperature -40\(^{\circ}\text{C}\), pH- 8.0, substrate concentration -1.5 \% w/v and inoculum size - 3.9887 \% v/v.

(iii) Rice husk (RH): Incubation period - 50.690 h, temperature- 42.402 \(^{\circ}\text{C}\), pH- 8.1681, substrate concentration -1.4159 \% w/v and inoculum size - 4.1689 \%v/v.

The optimum conditions of the most significant factors obtained for maximum production of keratinase enzyme using \textit{S.griseus} with various substrates are given below:

(i) Chicken feather powder (CFP): Incubation period -70.270 h, temperature -53.603, initial pH - 9.3603 , substrate concentration -1.5120\% w/v and inoculum size -3.9759 \% v/v.

(iii) Rice husk (RH): Incubation period -77.189 h, temperature -51.201°C, initial pH -9.985, substrate concentration -1.8243% w/v and inoculum size -4.168171 % v/v.

The kinetics of keratinase enzyme production was studied under optimum conditions using the optimized media using *B. subtilis* and *S. griseus* with CFP as substrates. Various models were tried to study the growth kinetics of *Bacillus subtilis* NCIM 2724. Logistic model was found to be the best model to predict the growth kinetics of microorganism *B. subtilis*. Modified Logistic incorporated Luedeking- piret model was found to be the best model to predict the product formation kinetics. Modified logistic incorporated Modified Luedeking - piret model was found to be best model to predict the substrate utilization kinetics.