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# Statistical analysis and mathematical modeling of potential microbial enzymes keratinases and pectinases for pharmacological benefits: A review

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**ABSTRACT**

*This research study originates from the recent reports how statistics and modeling plays a significant role that establish the viable scenario in modern microbial technology, Microbes like Actinomycetes and bacteria producing extracellular products known as Keratinases and pectinases are biocatalysts which possesses extraordinary specificity, remarkable catalysis power and having enormous pharmacological benefits. They are tremendous and potential to chemical catalysis due to their environmentally friendly character, as against traditional chemical methods, Microbial metabolic processes generally yield products and reduce the use of polluting chemicals by their exploitation in industry with the advancement of scientific and industrial developments Statistical analysis and mathematical modeling regarding microbial exploitation for the production of these potential enzymes is highly attractive for applications and treatment of Keratin ( hair and poultry) and fruit effluents .*

**Key words:** Keratin, Pectin, Pectinases, Keratinases, statistics and Mathematical modelling

## INTRODUCTION

Tirumala hills are a famous pilgrim center in Andhra Pradesh. It is located in Eastern Ghats on Seshachalam hill range, with North latitude of 13-14' and East longitude of 70-21'. It is 2820 feet above the sea level and 100 square miles in extent. Tirumala is being visited by thousands of pilgrims every day from all parts of India as well as whole world, all round the year to worship Lord Venkateswara{1,2,6,8}. The place has relatively high deposits of keratin as compared to any other place as most of the pilgrims coming to Tirumala consider head tonsuring as the most sacred act of offering to God. Further the prevailing climatic conditions also facilitate the growth of thermo-tolerant organisms. Thus this place was presumed to provide good enrichment for potential keratinophilic and keratinolytic organisms and soil samples collected from various locations in Tirumala Hills and Tirupati were analyzed to isolate keratinolytic organisms and Vellore district faces the problem of acute pollution due to tanneries in the adjoining areas. For every ton of raw hide processed, the amounts of solid waste produced are about 450-600 kg. About half of this contains 3% chrome on a dry matter basis. Loads of untanned hide by-product (e.g. fleshings, trimmings, splits etc from beam house is obtained which may lead to pathogen outburst. This vast amount of waste affects maximum the rural populace living in the area{5,4,3,9}. A number of Common Effluent Treatment Plants have been set up in the area, but less has been done to dispose off the tannery waste and favor the rural population in an integrated fashion. Microbial Pectinases are a group of enzymes responsible for hydrolysis of pectin and breakdown complex polysaccharides of plant tissues into simpler molecules with extra ordinary specificity, catalytic power and substrate specificity the study regarding statistical analysis and mathematical modeling of microbial enzymes is almost rudimentary in India hence the present study is carried out in this direction{7,9}

## MATERIALS AND METHODS

Statistics is the study of the collection, organization, analysis, interpretation, and presentation of data and it deals with all aspects of this, including the planning of data collection in terms of the design of surveys and experiments and a mathematical model usually describes a system by a set of variables and a set of equations that establish relationships between the variables. Variables may be of many types; real or integer numbers, Boolean values or strings, for example. The variables represent some properties of the system, for example, measured system outputs often in the form of signals, timing data, counters, and event occurrence. The actual model is the set of functions that describe the relations between the different variables. Media designing is an essential step in development of a fermented product which requires the selection of optimum nutritional and physical factors supporting the growth and production of desired product from the selected organism. Optimisation of parameters is done either by classical or statistical method In classical method for selection of media ingredients and fermentation parameters a uni-dimensional search is conducted wherein one nutrient or factor is changed each time by keeping all the other parameters constant. The optimized concentrations of the variable factor of the previous experiment are then incorporated in the next experiment and the same procedure is adapted for all the other parameters to obtain complete optimization .The limitations of the method include arbitrary selection of the initial concentration of constant variables, complete disregard to the interactions among different nutrients, requirement of much higher time, labour as well as cost intensive nature {10.11.12}

Several statistical designs, ranging from two factorial to multi factorial designs are available which overcome some of the above limitations In the multifunctional design, the experiments involved are $2^n$, where “n” is the number of ingredients. As the nutrients/ ingredients needed to be screened and optimized are more in the fermentation process, the experiments based upon multi-factorial design are complicated and difficult. Hence optimization of fermentation media is generally done at two step level, where initially the effect of the ingredients/nutrients are selected and further worked out for their optimized levels When the sources are many, it becomes some what difficult to place all the interactions when paired, as the ANOVA yields overall variation on the target variable with sources. In these cases, a multinominal logistic model is viable which gives relative efficiency of one variable over the other. Further the Tukey model of univariate analysis gives the identical subsets whose mean values are similar for identifying the best combination of the factors for selection. Along with the optimisation of nutritional requirements, optimization of physical parameters is also essential for developing efficient and economic fermentation process.{13}

### INDEPENDENT VERSUS DEPENDENT OBSERVATIONS

In addition to the nature of the variable being studied, the appropriate choice of statistical methodology is a function of whether the comparisons are made between independent or dependent experimental units. If two or more samples of experimental units are to be compared and the experimental units in one sample are not used again in the other sample, the observations in each experimental sample are independent of one another unless the units in one sample have been matched on a one-to-one basis with the units in the other sample. Typically, this matching is done on factors that are related to the response variable. These designs are known as paired or matched studies. When more than two measurements are made on the same experimental unit, the study is called a repeated measures design.

### ESTIMATION VERSUS TESTING, ERROR RATES, AND STATISTICAL POWER

It is important for an investigator to understand the difference between estimation and hypothesis testing. In almost every experiment, one of the goals is to determine the value of a true underlying population characteristic, for example, the median time to culture positivity of specimens from patients infected with a given organism. This is a process called estimation. In addition to the estimate of the true population parameter, it is common practice to also give 95% confidence intervals for the parameter..

### STATISTICAL HYPOTHESIS TESTS

The following subsections describe statistical methodologies that are usually appropriate for the given experimental situations. The reader should be aware that these are only general guidelines and that in many cases more sophisticated methods should be used. When there is any doubt about the appropriate method of analysis in an actual experiment, or of appropriate sample selection or appropriate methods to handle missing data, the aid of a professional statistician should be enlisted. Examples will be given for some (but not all) methods.

**Continuous Gaussian data.** When hearing in patients is measured in decibels before and after treatment with an antibiotic, the paired test (18) would be appropriate if the distribution of change in decibels is well behaved with no appreciable skewness and no outliers.

**Continuous non-Gaussian data.** If, in the previous example, the distribution of change in decibels was appreciably non-Gaussian, a more appropriate test than the paired t test would be Wilcoxon's signed-rank test.

**Statistical aspects of Microbiological sampling plans:**
The decision making process of a two-class plan is essentially defined by two numbers. The first, denoted as n, determines the number of sample units that are to be drawn independently and randomly from the lot. The second number, denoted as c, is the maximum allowable number of sample units yielding unsatisfactory test results, for example, the presence of the organism. In case of a two-class plan applied to grouped quantitative data there is one microbiological limit, denoted by m, which separates good quality from non-acceptable or defective quality. In this case the maximum allowable number of sample units exceeding this limit is given by c, which is usually set to zero for microbes which produces potential enzymes.

To visualize and study the performance of a sampling plan a graphical representation of its Operating Characteristic (OC) curve or function is useful. For a two-class plan this curve has two scales, the horizontal scale showing a measure of lot quality ike the fraction or percentage of positive ("defective") units in the lot being tested, the vertical scale giving the probability of acceptance., for example, depicts acceptance probabilities for lots in relation to the fraction of defective units when a two-class plan is applied specifying that a number of n=5 sample units are to be drawn and none of them (c=0) are allowed to be positive.

If evaluated for lots containing proportions defective that are regarded as not acceptable, for instance in a risk analysis context, acceptance probabilities characterize the risk that non-conforming lots will be falsely accepted. On the other hand, rejection probabilities, or one minus the according acceptance probabilities, that are derived for actually conforming lots describe the so-called producer's risk.

In situations where decisions are not based on results of presence-absence tests but on quantitative analytical results, three-class plans can be applied as an alternative to two-class plans working with data grouped according to a single microbiological limit for pharmacological usage.

**Classifying mathematical models**
Many mathematical models can be classified in some of the following ways:

1. **Linear vs. nonlinear:** Mathematical models are usually composed by variables, which are abstractions of quantities of interest in the described systems, and operators that act on these variables, which can be algebraic operators, functions, differential operators, etc. If all the operators in a mathematical model exhibit linearity, the resulting mathematical model is defined as linear. A model is considered to be nonlinear otherwise. The question of linearity and nonlinearity is dependent on context, and linear models may have nonlinear expressions in them. For example, in a statistical linear model, it is assumed that a relationship is linear in the parameters, but it may be nonlinear in the predictor variables. Similarly, a differential equation is said to be linear if it can be written with linear differential operators, but it can still have nonlinear expressions in it. In a mathematical programming model, if the objective functions and constraints are represented entirely by linear equations, then the model is regarded as a linear model. If one or more of the objective functions or constraints are represented with a nonlinear equation, then the model is known as a nonlinear model.

Nonlinearity, even in fairly simple systems, is often associated with phenomena such as chaos and irreversibility. Although there are exceptions, nonlinear systems and models tend to be more difficult to study than linear ones. A common approach to nonlinear problems is linearization, but this can be problematic if one is trying to study aspects such as irreversibility, which are strongly tied to nonlinearity.

2. **Deterministic vs. probabilistic (stochastic):** A deterministic model is one in which every set of variable states is uniquely determined by parameters in the model and by sets of previous states of these variables. Therefore, deterministic models perform the same way for a given set of initial conditions. Conversely, in a stochastic model, randomness is present, and variable states are not described by unique values, but rather by probability distributions.

3. **Static vs. dynamic:** A static model does not account for the element of time, while a dynamic model does. Dynamic models typically are represented with difference equations or differential equations.

4. **Discrete vs. Continuous:** A discrete model does not take into account the function of time and usually uses time-advance methods, while a Continuous model does. Continuous models typically are represented with f(t) and the changes are reflected over continuous time intervals.{14}

5. **Deductive, inductive, or floating:** A deductive model is a logical structure based on a theory {15}

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