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One way analysis of variance of temperature for efficient Pharmaceutically exploited microbes used in Bioremediation

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Abstract:
Objective: The present study was aimed at a One way ANOVA, statistical tool that were designed for various optimum temperatures has been adopted.
Methods: The One way ANOVA method was used to confirm the optimum temperature which is necessary for a microorganism to survive and function with maximum efficiency.
Results: One parameter analysed here is the temperature at which the microbes survive. Eight different microbes have been taken into consideration here.
Conclusions: This study conclude that One way ANOVA, statistical tool that helps in getting the optimum results for the growth of microbes that make the regulation of parameters easy, which are being produced and customized to increase efficiency, reduce cost, time and even man-power.

Keywords: Bioremediation, Temperature, One way ANOVA, Soil microbes

Introduction

Pharmacy and pharmaceutical sciences are recently emerging technologies which solves human health problems. In this study we want to highlight the statistical analysis of pharmacy related microbes with biodegradation potential. Bioremediation is the biological technique where microorganisms are utilized in the degradation of organic or even inorganic pollutants. Recalcitrant, xenobiotic and even heavy metals can be degraded by using a variety of microbes available in the environment. Bioremediation is further classified into in situ bioremediation and ex situ bioremediation. In situ deals with treatment of the waste within the site while ex situ deals with removing waste and treating them at another site. The main aim of bioremediation is to degrade the pollutants in the environment that pose as a major threat to human health.

The basic understanding of the mechanism involved in degradation is that these contaminants are used as nutrients or carbon and energy sources by the microorganisms [1]. Bacteria, fungi and various protozoans are used for the breakdown due to their unique properties. Phytoremediation is also widely used. Various microbes are also being genetically modified to suit the requirements. The major advantage of bioremediation is that it is inexpensive, less-technologically oriented and can easily be carried out on site [2].

Microbes used

The various microbes involved in bioremediation vary over a variety of species and there related

genus and can also be classified into aerobic and anaerobic. Aerobic microorganisms include *Pseudomonas*, *Alcaligenes*, *Sphingomonas*, *Rhodococcus*, and Mycobacterium while anaerobic microorganisms include Ligninolytic fungi (*Phanerochaetechrysosporium*) and methylotrophs [3].

Pseudomonas putida

Pseudomonas putida is classified as a gram-negative bacterium that is rod shaped and is flagellated. Being aerobic,its natural habitat is usually soil and water where abundant amounts of oxygen are present. It survives at an optimum temperature of 26°C and at a pH of 6.5-7. KT2440 is the most abundantly used strain to colonize plant roots and establish a relationship between the plant and the soil.The action of *P.putida* is to degrade organic solvents and its major role is to convert styrene oil into a biodegradable plastic, Polyhydroxyalkanoates (PHA). The process of styrene degradation by *Pseudomonas putida* CA-3 to degrade styrene can be carried out by two different pathways being: vinyl side chain oxidation and attack on the aromatic nucleus of the molecule [4].

Dechloromonasaromatica

Dechloromonasaromatica are gram-negative, rod shaped bacterium and are found in aquatic settlements. They can oxidize aromatic compounds such as toluene, benzoate, and chlorobenzoate. The optimum growth temperature for this organism is 30°C and is the only organism that is capable of oxidising benzene anaerobically. In laboratory conditions, it is stored in an environment of 80% nitrogen and 20% carbon dioxide. Benzene is highly soluble, mobile, toxic and stable in ground and surface waters.

Nitrosomonaseuropaea

Nitrosomonaseuropaea is a gram-negative microbe which is also a chemolithoautotroph shaped as a bacillus. It survives at an optimum temperature of 26°C and is aerobic. As evident from its name, *N.europaea* is present in the soil and thrives in other places rich in ammonia salts and inorganic salt present in sewages, freshwaters, etc. It is often found in the atmosphere of polluted areas where the air contains great levels of nitrogen compounds and its derivatives. *N.europaea* degrades a variety of halogenated organic compounds that include trichloroethylene, benzene, and vinyl chloride. The property possessed by nitrifying organisms to degradepollutants makes these microbes attractive for controlled bioremediation in nitrifying soils and waters. Conversion of ammonia in the wastewater to dinitrogen is commonly tried by using *Nitrosomonaseuropaea* as it contains enzymes that oxidise ammonia such as nitrite reductase and nitrous oxide reductase.

Nitrobacterhamburgensis

This organism represents another class of soil microbes involved in nitrogen fixation. *N.hamburgensis* is a gram-negative bacterium that thrives on soil, sandstone and sewage sludge. It functions at a temperature of 38°C and at a neutral pH of about 7.3-7.5. *N.hamburgensis* is an example of nitrite-oxidizing bacteria and hence is aerobic. The bacterium has provided a solution to remove high levels of nitrogen from municipal effluents of wastewater treatment plants.

Paracoccusdenitrificans

This organism is a gram-negative coccus and it can live in both aerobic and anaerobic conditions. They survive at a temperature of 26°C. *Paracoccusdenitrificans* can produce more than 5000 different proteins. An application of one such

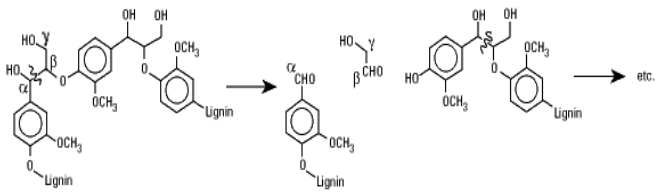
protein is in the construction of a bioreactor that helps in the eradication of nitrogen from wastewater. *P.denitrificans* has the unique ability to reducenitrite into nitrogen gas. The system of *Paracoccusdenitrificans* with *Nitrosomonaseuropaea* simplifies the process of the removal of nitrogen from wastewater and hence helps in the degradation of organic pollutants.

Phanarochaetechrysosporium

Commonly known as the white rot fungus, it is a ligninolytic fungus. It derives its name from the phenomenon where the aromatic polymer lignin is degraded and leaves the white cellulose untouched thus leaving to white depositions on the surface. *Phanerochaetechrysosporium* releases enzymes that are extracellular in nature. They help in breaking up the complex three-dimensional structure of lignin into independent components that are then utilized for their own metabolisms. These extracellular enzymes are non-specific in nature and they are oxidizing agents (hydrogen peroxide and hydroxyl radicals) that are used in the cleavage of lignin bonds. *P.chrysosporium* can be found in aerobic conditions under temperatures ranging from 20-25°C.

Figure 1

Lignin breakdown



The process of lignin breakdown is a cleavage reaction. The extracellular enzymes secreted by the organism releases free-radicals that initiate an immediate break down to phenyl propane units in the secondary metabolism mechanism.

Deinococcusradiodurans

Deinococcusradiodurans is a peculiar gram-positive bacterium with a spherical tetrad structure and is named so due to its ability to endure large amounts of radiation that can easily kill a human being. This organism has been isolated from many regions including soil and faeces. It is a mesophile and hence survives at a temperature range from 30-37°C in a neutral environment (pH 7). It is an aerobic organism. Scientists are particularly interested in using this bacterium to clean up waste sites that are polluted with hazardous materials. *D.radiodurans* is known to be able to break down solvents such as toluene from olden times however, improvements need to be mediated to try and make the bacterium capable of breaking down other components and materials that are extremely common at radioactive waste sites which could help the environment in unmeasurable ways and even promote efficient disposal of radioactive wastes that is currently a major health and environment hazard [7].

Parameters of Microbes to be taken into account

On a commercial scale, when microbes are taken into account, various parameters are considered before the right one is chosen for usage. Not only must the microbe be monitored but also the soil that is to be treated also needs to be analysed to choose the correct microbe. The most common parameters taken into account are temperature, pH, pressure, humidity and the concentration of oxygen that needs to be present. Most microbes used in bioremediation are aerobic. In some cases, anaerobic microbes are also used especially for the degradation of aromatic

compounds such as benzene. When considering the combined parameters of soil and microbe, various parameters can be considered for estimation.

Table 1: Parameters of the soil and microbes that are considered for bioremediation [6]

CLASS OF FACTOR	MEASURABLE FACTOR	EXPRESSION
Kinetic	• Activity of indigenous organisms • Presence of inhibitory or toxic compounds	• k, χ, Km • Inhibition or activity decay constant
Equilibrium	Partitioning of contaminants	Partition coefficients
Transport	• Desorption rates • Oxygen supply • Nutrient supply	• ksAs • klA

Microbial growth, oxygen supply, nutrient consumption, etc. are other parameters that are taken into consideration when the thermodynamic aspect of bioremediation needs to be estimated in terms of choosing the right microbe.

To determine whether a parameter has an effect on the microbe or not, one uses statistical calculations. On considering the data of each microbe and comparing them by Analysis of Variance (ANOVA), a hypothesis can be made as to how important a parameter is and the intensity with which it affects the microorganism. The variance can be calculated as one way, two way, three way, etc. depending on the number of parameters considered.

MATERIALS AND METHODS

To prove that an optimum temperature is necessary for a microorganism to survive and function with maximum efficiency, the one way ANOVA method can be used to bring validity to this proof on a mathematical/statistical platform.

For this, two contradicting hypotheses need to be assumed,

H_0: The microbes require an optimum temperature for survival ($F_c<F_t$)

H_1:The microbes do not require an optimum temperature for survival ($F_c>F_t$)

Now, the range of temperatures in which the microbe can survive can be taken in 3 limits as minimum, optimum and maximum temperature. As a result, three replicates are required and hence r = 3.

Column 1 represents the minimum temperature (°C).

Column 2 represents the optimum temperature (°C).

Column 3 represents the maximum temperature (°C).

Table 2: Table of data

Replicates →	1	2	3
P.putida	25	26	30
D.aromatica	25	30	30
N.europaea	25	26	30
N.hamburgensis	20	38	49
P.denitrificans	24	26	28
P.chrysosporium	20	25	25
D.radiodurans	25	27	37

'n' for all the rows are 3.

On calculating for each row,

Table 3: Σx

Σx	81	85	81	107	78	70	89

On calculating the $\bar{x}$,

Table 4: $\bar{x}$

$\bar{x}$	27	28.3	27	29	35.67	23.3	29.67

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Table 5: Σx^2

Σx^2	2201	2425	2201	4245	2036	1650	2723

$(\Sigma x)^2 / n$

Table 6: $(\Sigma x)^2 / n$

$(\Sigma x)^2 / n$	2187	2408.3	2187	2523	2028	1633.3	2640.3

$\Sigma d^2 = \Sigma x^2 - [(\Sigma x)^2 / n]$

Table 7: Σd^2

Σd^2	14	16.7	14	428.7	8	16.7	82.7

On calculating the variance [$\sigma^2 = \Sigma d^2/(n-1)$]

Table 8: σ^2

σ^2	7	8.35	7	214.35	4	8.35	41.35

F_{max} (calculated) = $\sigma^2_{max} / \sigma^2_{min}$ --- Variance ratio
F_c = 214.35 / 4 = 53.58

On looking up the value from the F_{max} table, with the number of treatments from the table of data being 3 and the degrees of freedom (defined as the number of replicates per treatment – 1) as 2, we obtain a value of 87.5.

Since $F_c < F_t$, the calculations can be carried on.

A = Sum of all the values of Σx^2 = 17481

B = Sum of all the values of $(\Sigma x)^2 / n$ = 15606.9

D = (grand total)^2 / total number of observations = $(591)^2 / 9$ = 38809

Total sum of squares = A-D = 17481-38809 = -21328

Between treatments sum of squares = B-D = 15606.9-38809 = -23202.1

Residual sum of squares = A-B = 17481-15606.9 = 1874.1

Table 9: ANOVA table

Source of Variance	Sum of Squares (S of S)	Degrees of freedom (df)	Mean square (S of S / df)
Between treatments	-23202.1	2	-11601.05
Residual	1874.1	6	312.35
Total	-21328	9	

F_c = between treatments mean square / Residual mean square

F_c = -11601.05 / 312.35 = -37.14

F_t = 5.1 (u=2, v=6) ---------- F (p=0.05) table

Therefore, $F_c < F_t$

Hence the null hypothesis is accepted stating that the microbes require an optimum temperature to function efficiently under the prescribed conditions. Also there is not much of a significant difference between the treatments (optimum temperatures).

Results

One way ANOVA is only one simple analysis technique that can be used to statistically prove the importance of parameters. pH, pressure, humidity, oxygen content, nutrient consumption, growth rate, etc. represent only a small portion of parameters that need to be considered. Soil parameters are first determined and then based on them the microbial parameters are then determined. To make the regulation of parameters easy, genetically engineered microbes are being produced and customized to increase efficiency, reduce cost, time and even man-power. *Deinococcusradiodurans* is an example of such a microbe where extensive research is being carried out to improve itscharacteristics.

Bioremediation in itself is a very vast field. Under this, many other technologies have been adopted such as phytoremediation, bioaugmentation, bioventing, bioleaching, landfarming, composting, biostimulation and rhizofiltration. Modern bioremediation does not just restrict itself to the use of microorganisms. It was found that certain fish bones were capable of

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absorbing some amounts of lead from contaminated soils [7]. Also bone char is used nowadays to bioremediate cadmium, silver and copper [8].

It can be safe to say that the process of bioremediation is a natural and ancient one where no external or human force is required to mediate the process. Depending on the conditions of the habitat where the microbe is present, the microbe adapts itself and deploys a mechanism of action to degrade any foreign material that is toxic to the environment. In other words the microbe uses its defence mechanism to save itself however in the process it is unknowingly protecting the environment too. This is why the microbes used currently are known to be indigenous. Researches on non-indigenous microbes are also being carried out as a branch of genetic engineering.

Conclusion

Analysing the different temperatures and then selecting a specific optimum temperature can help to design an efficient system for bioremediation. Bioremediation on the whole is an ancient method of waste degradation and many attempts to improve this technique is being carried out for this. The one way Analysis of Variance is a statistical technique used to decide the optimum temperature. Many such parameters can be considered and analysed. The combination of statistics and life sciences is not a new venture. Many more comparisons can be done and this can help to improve researches and even quality of waste treatments in the near future.

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References

1) Jacquelyn G. Black, Laura J. Black, 2008. Microbiology Principles and Explorations, 7thEdition. John Wiley & Sons, Inc. pp. 809
2) Madhavi Gaonkar Naik and Mohini Dilip Duraphe. "Parameters Affecting Bioremediation". International J of Life Sci and Pharm Res, Vol2/Issue 3, July-September, 2012.L-77.
3) Madhavi Gaonkar Naik and Mohini Dilip Duraphe. "Parameters Affecting Bioremediation". International J of Life Sci and Pharm Res, Vol2/Issue 3, July-September, 2012.L-78.
4) O'Connor, K.,Duetz, W., Wind, B., Dobson, A. D. W. "The Effect of Nutrient Limitation of Styrene Metabolism in Pseudomonas putidaCA-3". App and Env Microbiol. October 1996. Volume 62.p.3594-3599.
5) DeWeerdt, S. E. The World's Toughest Bacterium. Genome News Network, July 5, 2002.
6) S.C. Bhatia. Handbook of Env Microbiol, Vol. 3.Atlantic Publishers & Distributors (P) Limited, 2008.
7) Kris S. Freeman. "Remediating Soil Lead with Fishbones". Env Health Perspect. January 2012.
8) Huan Jing KeXue. "Chemical fixation of metals in soil using bone char and assessment of the soil genotoxicity". February 2007.
9) Suneetha V Statistical analysis and mathematical modeling of potential microbial enzymes keratinases and pectinases for pharmacological

benefits: A review Der Pharmacia Lettre Volume 6, Issue 2, April 2014, Pages 169-172.

10) R. Gopinath and Suneetha V. Taguchi's - A Recent Statistical Approach to Optimize the Microbial Pectinases Screened from Fruit Waste Yard, Journal of pure and app microbiol, 2014, Volume 8 No. 2 Page No. 1749-1754.

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