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Statistical optimization of process variable for degradation of pulp paper mill effluent

Yogesh Tandan and Amar Abhishek

Abstract
The paper industry is characterized by the high rate of water consumption, so high rate of wastewater produced. In India lignin was used extensively by the paper and forestry industry to prevent sap staining of wood. Lignin affects the living organism due to their toxicity. Microorganism are nature's innovative recyclers, converting toxic organic compound to harmless species. Bacteria seems to be more effective than fungi for the bioremediation of environmental pollutants due to their immense environmental adaptability and biochemical versatility.
The outcome of the any biological treatments depends upon a numerous parameter such as pH, temperature, incubation time, carbon source and agitation speed. The conventional orthogonal approach (one-factor at a time) for optimization of the process parameters requires a very large number of experimentations, which would be very expensive and time consuming. To overcome these drawbacks, one of the statistical design tools, called Response surface methodology (RSM), can be used for the process optimization and prediction of interaction between several process variables. RSM is a collection of statistical technique for designing experiments, building models, evaluating the effects of factor and searching for the optimum conditions.

Keywords: Statistical optimization, RSM, good grade paper, lignin

Introduction
In order to manufacture good grade pulp in pulp paper industry, only high- quality Fiber containing wood are preferred, with an extra chemical process involving extensive prehydrolysis of wood chips at elevated temperature and pressure followed by alkaline digestion. This process ensures the removal of waste materials and remaining fibers with high cellulose content. A significant number of organic compounds present in pulp-paper mill effluent have been classified as mutagenic and clastogenic thereby turning these effluents into 'a Pandora's box of waste chemicals' (Pokhrel and Viraraghavan 2004) [1]. Some of these compounds are naturally occurring (mainly lignin & its derivatives), and others are formed during the process of paper making (chlorinated lignin's, resin acids and chlorinated phenols, dioxins, furans) (Zaied and Bellakhal 2009) [4]. In most cases, this effluent (row or treated) is discharged into the rivers, stream or other water bodies; resulting in negative social and environmental impacts (Kreetachat *et al*. 2007; Chandra *et al*. 2011) [2, 13]. Thus, it is obligatory to treat the effluent before disposal. Despite the fact that, several physical and chemical methods are available for the treatment of effluent, but they are less desirable than biological process because of cost-ineffectiveness and residual effect (Singhal and Thakur 2009) [3].
Microorganisms are nature's innovative recyclers, converting toxic organic compounds to innocuous species. The biological methods tried so far, most of the literature is confined to a few genera of white rot fungi. But bacteria seem to be more effective than fungi for the bioremediation of environmental pollutants due to their immense environmental adaptability and biochemical versatility (Raj *et al*. 2007; Das *et al*. 2012) [9, 6]. However, the bacterial treatment studies have confined themselves to the evaluation of microorganism, basic mechanism behind the treatment, detection and optimization of ligninolytic enzyme (Jain *et al*. 1997; Singh *et al*. 2007; Ko *et al*. 2009) [5, 8]. Although, decolorization of pulp paper effluent is well reported by pure culture (Morii *et al*., 1995; Jain *et al*., 1997; Gupta *et al*. 2001; Das *et al*. 2012) [5-7], the results of such studies are not necessarily relevant to the field because microorganisms in nature grow mostly in mixed condition.

The outcome of the any biological treatments depends upon numerous parameters such as pH, temperature, incubation time, carbon source and agitation speed. The selection of optimal operating conditions is case-specific, and may lead to effective removal of pollutants. But unfortunately, rarely any research has been done in this regard. The conventional orthogonal approach (one-factor at a time) for optimization of the process parameters requires a very large number of experimentations, which would be very expensive and time consuming. To overcome these drawbacks, one of the statistical design tools, called Response surface methodology (RSM), can be used for the process optimization and prediction of interaction between several process variables. RSM is a collection of statistical techniques for designing experiments, building models, evaluating the effects of factors and searching for the optimum conditions. The most important part before applying the RSM methodology is the selection of appropriate design of experiment. It has a large influence on the building of response surface and thus, on the precision of its prediction. In the present study, a central composite design (CCD) which is widely used form of RSM was employed for the optimization of bacterial decolorization of pulp paper mill effluent.

Review of Literature

Pulp and paper are manufactured from raw materials containing cellulose fibers, generally wood, recycled paper, and agricultural residues. In developing countries, about 60% of cellulose fibers originate from nonwoody raw materials such as bagasse (sugar cane fibers), cereal straw, bamboo, reeds, esparto grass, jute, flax, and sisal. This document addresses environmental issues in pulp and paper manufacturing with unit production capacities greater than 100 metric tons per day (tpd) (Chandra and Abhishek, 2011) [2]. The main steps in pulp and paper manufacturing are raw material preparation, such as wood debarking and chip making; pulp manufacturing; pulp bleaching; paper manufacturing; and fiber recycling. Pulp mills and paper mills may exist separately or as integrated operations. (Kumar *et al*., 2019; Khan *et al*., 2022) [12, 11]. Manufactured pulp is used as a source of cellulose for fiber manufacture and for conversion into paper or cardboard (Chandra *et al*., 2007) [13] Pulp manufacturing starts with raw material preparation, which includes debarking (when wood is used as raw material), chipping, and other processes such as depithing (for example, when bagasse is used as the raw material) (Brad Carlberg, 2020) [10] Cellulosic pulp is manufactured from the raw materials, using chemical and mechanical means. The manufacture of pulp for paper and cardboard employs mechanical (including thermomechanical), and chemical methods.

From financial point of view, pulp and paper production is a considerable source of income for the manufacturing countries. On the other hand, however, the extension of this industry provokes serious concern because of the forest exhaustion and the very slow rate of their restoration. That is why some countries, like Norway, prefer to import wood for paper industry, instead of use of their own. Brazil for example has started a program for planting of rapidly growing trees for this purpose. It is clear therefore, that paper industry and the consumption of paper have impact on environment not only through the pollution that the paper production enterprises exert on air, water and soils, but as well as to a higher extent by the deforestation that result for paper demand and production. At various stages of the process, chemicals are used to give the paper particular properties, such as the bleaching chemicals that make paper white (and which also enable it to subsequently be coloured). The pulping process that is used in New Zealand is known as "kraft pulping" which relies on a combination of heat, chemicals and mechanical pulping to convert the wood into a smooth, soft pulp suitable for use in paper making. Kraft pulping is the main pulping process (together with mechanical pulping) used today, and is the only one discussed below.

In pulp paper industry following type of wastes release

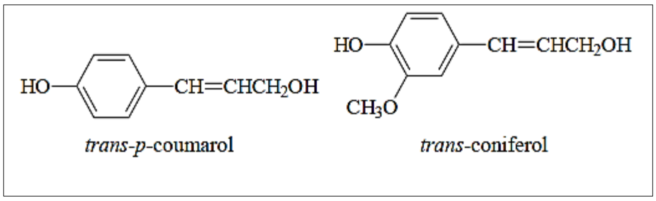
In the Pulp and Paper Industry several types of solid wastes and sludge as well as wastewater are generated. About 40-50 kg of sludge (dry) is generated in the production of 1 ton of paper at a paper mill and of that approximately 70% is primary sludge and 30% secondary sludge. The amount of sludge on a dry mass basis may vary from 20% in a newsprint mill to 40% in a tissue mill. The data on waste generated in pulp and paper mills and deinking mills are presented in this chapter. Waste generated through production of different paper grades from recycled fibre are also presented. On the other hand, there are wastewater or liquid waste that contain many types of chemicals that is toxic for the environment as well as the human being and other living creatures and organism. The waste water contains lignin, pentachlorophenol, phenol, methyl ethyl ketone, acetone, alcohols, chloroform, sulfur compound, organochlorine compound, chloromethane and trichloroethane etc.

Natural Lignin (lignocellulose)

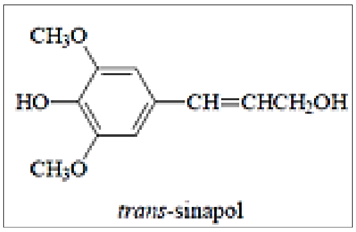
The plant contain wide variety of natural cellulosic materials has complex and uneven components. Cellulose, hemicellulose, and lignin comprise the main composition of cell walls of plants and are important components of natural lignocellulosic materials. Cell walls of plants consist mainly of three organic compounds: cellulose, hemicellulose, and lignin. These compounds are also major components of natural lignocellulosic materials. Cellulose molecules arrange regularly, gather into bundles, and determine the framework of the cell wall. Fibers are filled with hemicellulose and lignin. The structure of the plant cell wall is compact. There is different bonding among cellulose, hemicellulose, and lignin. Cellulose and hemicellulose or lignin molecules are mainly coupled by a hydrogen bond. In addition to the hydrogen bond, there is the chemical bonding between hemicellulose and lignin, which results in the lignin, isolated from natural lignocelluloses, always contains a small amount of carbohydrates.

Table 1: Chemical Composition and Structure of Natural Lignocellulose.

Waste	Yield (kg/t o.d. pulp)
Wood wastes	
Sawdust coming from the slasher deck	10-30
Bark falling from the debarking drum	100-300
Pins and fines from chip screening	50-100
Wood waste from woodyard	0-20
Knots from pulp deknotting	25-70
Sodium salts from recovery boiler	5-15
Dregs and grit from causticizing:	5-10
Dregs	10-30
Grit	15-40
Total	220-615



Lignin: The main component of wood that needs to be removed to turn it into paper is a compound known as lignin. This name refers to a group of chemicals that are essentially three-dimensional polymers of *trans*-coniferol, *trans*-sinapol and *trans*-*p*-coumarol (see below), along with hemicelluloses and aromatic carboxylic acids. Lignin is the reinforcing compound that is deposited on tree cell walls to make the wood strong enough to carry the weight of the tree crown. However, it is also the compound that makes wood pulp brown, so it is removed from all wood pulp except that used to make brown paper and some cardboards.



Lignin		Hemicellulose	Cellulose
Subunits	Guaiacylpropane (G), syringylpropane (S), p-hydroxyphenylpropane (H)	D-Xylose, mannose, L-arabinose, galactose, glucuronic acid	D-Pyran glucose units
Bonds between the subunits	Various ether bonds and carbon-carbon bond, mainly B-O-4 ether bond	B-1,4-Glycosidic bonds in main chains; B-1.2-, B-1.3-, B-1.6-glycosidic bonds in side chains	B-1,4-Glycosidic bonds
Polymerization	4,000	Less than 200	Several hundred to tens of thousands
Polymer	G lignin, GS lignin, GSH lignin	Polyxylose, galactoglucomannan (Gal-Glu-Man). glucomannan (Glu-Man)	B-Glucan
Composition	Amorphous, inhomogeneous, nonlinear three-dimensional polymer	Three-dimensional inhomogeneous molecular with a small crystalline region	Three-dimensional linear molecular composed of the crystalline region and the amorphous region
Bonds between three components	Contain chemical bond with hemicellulose	Contains chemical bond with lignin	Without chemical bond

Lignin is one of the most abundant organic polymers in plants, just behind cellulose. It is the exclusive chemical composition of gymnosperm and angiosperm. The content of lignin in wood and Gramineae is 20-40% and 15-20%, respectively. Lignin is the name of a group of substances; their inhomogeneity is manifested in different species of plants, length of growing season, and different parts of the plants. Even in the different morphologies of cells of the same xylem or different cell wall layers, the structures of lignin are not the same. Lignin is a complex composed of complicated phenylpropane units nonlinearly and randomly linked; three main monomers are coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol. Because of the different monomers, lignin can be divided into three types syringyl lignin polymerized by syringyl propane, guaiacyl lignin polymerized by guaiacyl propane, and hydroxy-phenyl lignin polymerized by hydroxy-phenyl propane. Usually, gymnosperm mainly contains guaiacyl (G) lignin; the dicotyledon mainly contains guaiacyl-syringyl (GS) lignin; the monocotyledon mainly contains guaiacyl-syringyl- hydroxy-phenyl (GSH) lignin. At one time, lignin in plant was divided into softwood, hardwood, and grass lignins. Based on the structure of lignin, Gibbs divided lignin into G lignin and GS lignin. G lignin is chiefly formed through dehydrated oligomerization of coniferyl alcohol, and its structure is homogeneous. This kind of lignin has negative Maule reaction because less than 1.5% of syringaldehyde and about 5% of p- hydroxybenzaldehyde were generated when oxidized by nitrobenzene. Most lignin in softwood belongs to G lignin, which is copolymerized by guaiacyl and has a positive Maule reaction. GSH lignin is the result of the dehydrated oligomerization of coniferyl alcohol and sinapyl alcohol; the content of lignin is 17-23%. The ratio of syringyl propane to guaiacyl propane is 1.5_0.1; it also contains 7-12% ester groups. p-Coumaryl alcohol in it is linked to lignin in the form of ester.

Materials and Methods

Work Place: The present dissertation work "Statistical Optimization of Process variable for degradation of pulp paper mill effluent" was carried out in the PG Laboratory, Department of Botany, Guru Ghasidas vishwavidyalaya, Bilaspur.

Chemicals and glass wares: The entire chemical compounds for various media preparation were provided from the laboratory. All the glass and plastic wares were cleaned and autoclaved under the steam pressure of 15 lbs at 121 ºC for 15 min. Solutions were made in distilled water and sterilized by autoclaving.

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Sampling site

For the isolation of potential bacteria, activated sludge was used. This sludge was collected from the main channel of pulp paper mill (paper industry of Raigarh). The samples were randomly collected in presterilized plastic bag in triplicate manner. Sample collection, transportation and preservation were carried out as per standard protocol given in APHA (American physiochemical health association), 2005.

Isolation of Bacterial Strains: For isolation of potential bacteria enrichment method was adopted. In this method, one loopful of activated sludge was transferred aseptically to sterile media containing 2000 mg/L lignin. The flasks were incubated for 10 days at 120 rpm at 300C.

Experimental design

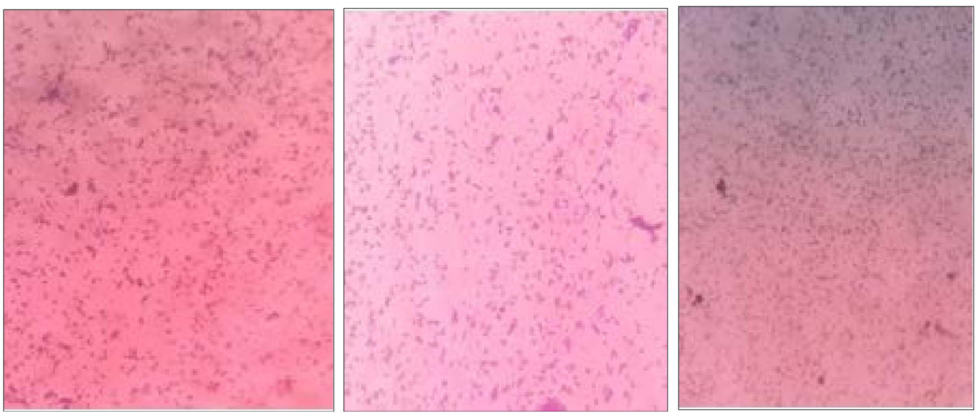
Experiments were conducted to choose the best carbon source (glucose, galactose, dextrose and fructose), Agitation speed; pH; temperature and incubation time. After selecting the three most influencing factor, Response surface methodology nbuilt with CCD was employed. The three parameters (factors) i.e. pH (XpH), Agitation (XAgi) and Carbon source (Xc) were investigated at five levels (-√2,-1, 0, +1, +√2) each to optimize the dependent response variables i.e.% decolorization (Y1). The study included 15 experiments with 5 runs at central level as replicates. A regression model was proposed, and results were analyzed using Design Expert software version 7.0 (Minitab Institute, USA). The responses (Y1 and Y2) were related to chosen variables by full second-order polynomial (quadratic) model. The second-order model in terms of coded variables can be expressed as follows:

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_1 x_2 + \beta_5 x_1 x_3 + \beta_6 x_2 x_3 + \beta_7 x^2{}_1 + \beta_8 x^2{}_2 + \beta_8 x^2{}_3 \quad (1)$$

Where 'Y' is the predicted response; β0 is intercept term, β1, β2 and β3 are first order linear coefficients; β4, β5 and β6 are the interaction coefficients; while β7, β8 and β9 are the second order quadratic coefficients. The analysis of variance (ANOVA) was performed based on the proposed model to find out interaction between the process variables and response. The quality of the fit for the polynomial model was expressed by the coefficient of determination (R2), and the statistical significance was checked by the F-value (Fischer variation ratio) and p-value (significant probability value). Finally, the 3-D response surface curves were plotted between the two factors, keeping the third factor fixed at the central level. The optimum requirement of each factor was identified by numerical optimization, i.e. desirability functions. All 15 experiments were conducted in triplicates to take the mean value.

Results and Discussion

Enrichment method is one of the most powerful techniques available to the microbiologist to isolate the bacteria from mixed culture from nature. An almost infinite number of combinations of the different environment factors for nutritional and physical can be developed for the specific isolation of microorganisms from nature. Nutrient enrichment technique specifies the isolation of specified bacteria from nature, by taking advantages of their specific nutrient requirements. In this work, we try to isolate lignin degrading bacteria which was useful for pulp paper mill effluent. For isolation of potential bacteria strains, activated sludge was add in MSM medium amended with only 2000 ppm lignin as sole carbon source. After 10 days 0.1 ml sample from the flask was spreads on Agar medium and incubated for 24 to 48 h. different bacterial colonies were picked and purified by repeated streaking on fresh medium. Based on different colony morphology total of three bacterial strains were isolated. However, purification of these bacterial by microscope revealed that out of eight strains, only three strains were found to be pure. Those three bacteria, one of them are gram positive bacteria(+ve), and rest of two bacteria are gram negative(-ve). All the three bacteria are the rod in shape.



A **B** **C**

Fig 1: A) gram -ve bacteria B) gram -ve bacteria C) gram +ve bacteria

Measurement of Growth pattern during the time course of degradation

During the time course of experiment, we pulled out some amount (5 ml) of culture supernatant every day for measurement of growth pattern of bacteria (at 600nm). Results revealed that, mixed culture was high growth rate compare to single one (Figure 2). This is may be due to supportive growth of bacteria in the presence of other one in syntrophic manner. This mixed culture was selected for further final statistical optimization of process variable.

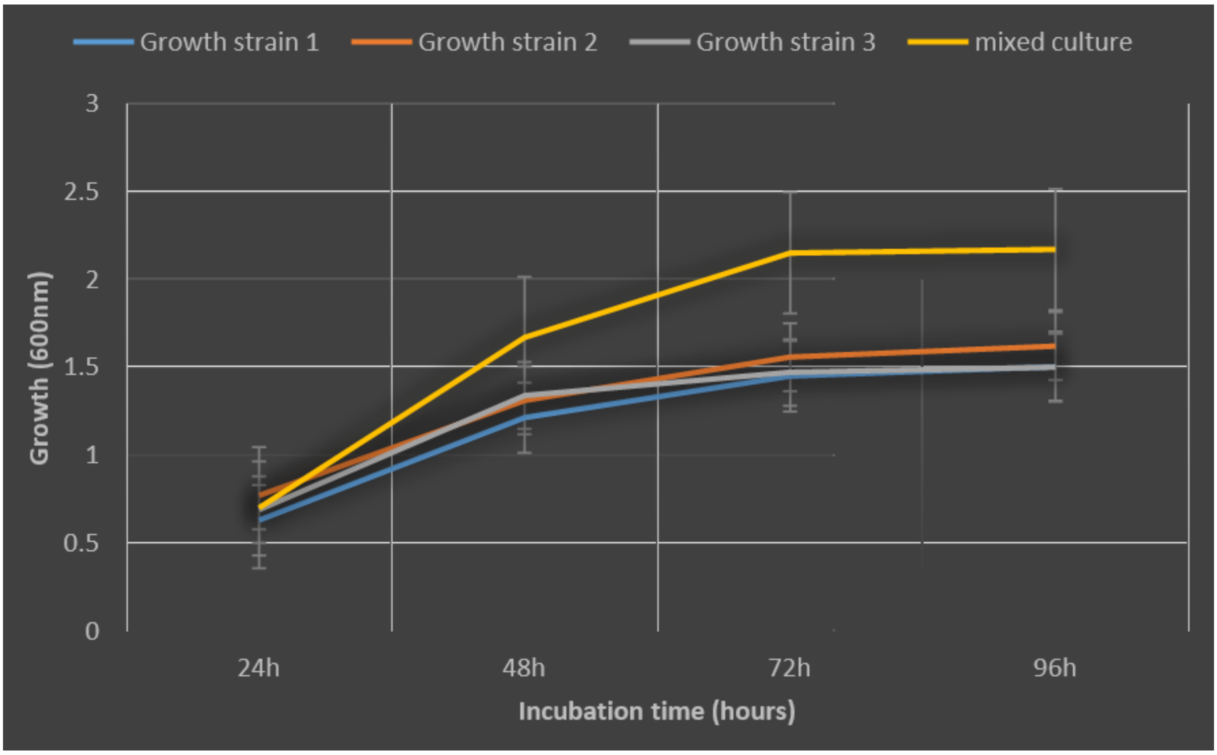


Fig 2: Growth pattern of individual and mixed bacterial culture

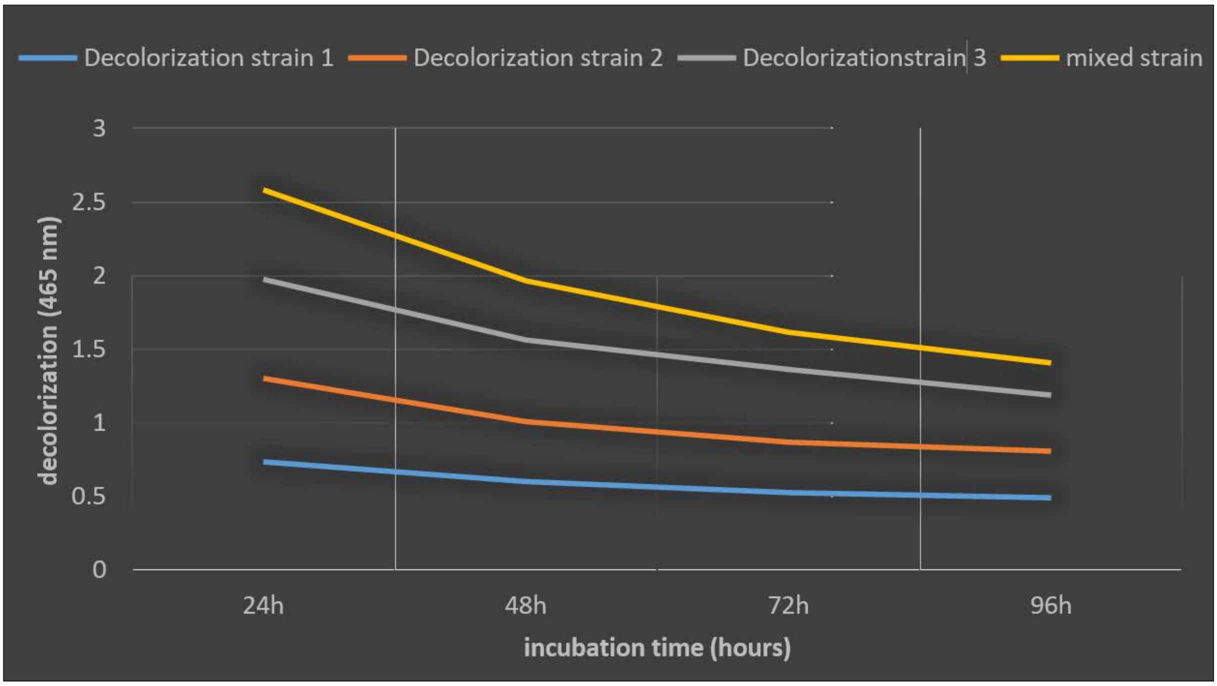


Fig 3: Decolorization pattern of individual and mixed bacterial culture

Fig 4: Growth and decolorization of single strains

Fig 5: Growth and decolorization of mixed strain

Optimization of effluent

Different kinds of optimization opportunities are available in the manufacturing plant. It either involves energy saving, chemical saving, increase the production rate and so on.

In the search of optimum condition, we used different factors i.e. carbon sources; incubation time (12-72h); pH (6-9); Agitation speed (100-300 rpm); and temperature (30-40 °C) was optimized by the predictable one factor in one time method. Glucose as a carbon source, pH (alkaline) and agitation speed (100-300 rpm) were found to be prominent factors for effluent treatment at temperature 32 °C and incubation time 72 h. This structural statistical design (RSM) is constructed and utilized for single factor along with their interactive effects with other factors on the reduction of lignin degradation effluent. Construction of skeleton in CCD design and their order effects (first and second order).

The optimum condition for the treatment of effluent is

- Carbon source 2.1%
- Agitation speed 235 rpm
- pH 7.8

These three conditions constructed the finest grouping for the improvement of decolorization up to 91% (Table 4.2). The statistical model was evaluated by ANOVA and gave regression equation for the% decolorization (Y1).

Y1=+68.31+2.24XpH+1.42XAgi+4.78XC-13.72XAgi-0.32XAgi2-.12X2+0.37XpHXAgi+8.81XpHXC-0.14XAgiXC

Table 1: Experimental design layout as per CCD along with the values of response variable.

S. No.	pH	Agitation speed (rpm)	Carbon source (%)	% x Decolorization
1.	8	200	0.59	70
2.	7	130	1	73
3.	8	180	2	87
4.	8	200	3.41	80
5.	8	180	2	88
6.	8	200	2	86
7.	7.8	235	2.1	91
8.	8	100	2	84
9.	7.5	100	2	72
10	7	170	3	73
11	8	200	2	83
12	8	200	2	84
13	9	130	3	78
14	6.59	200	2	72
15	8	110	2	78

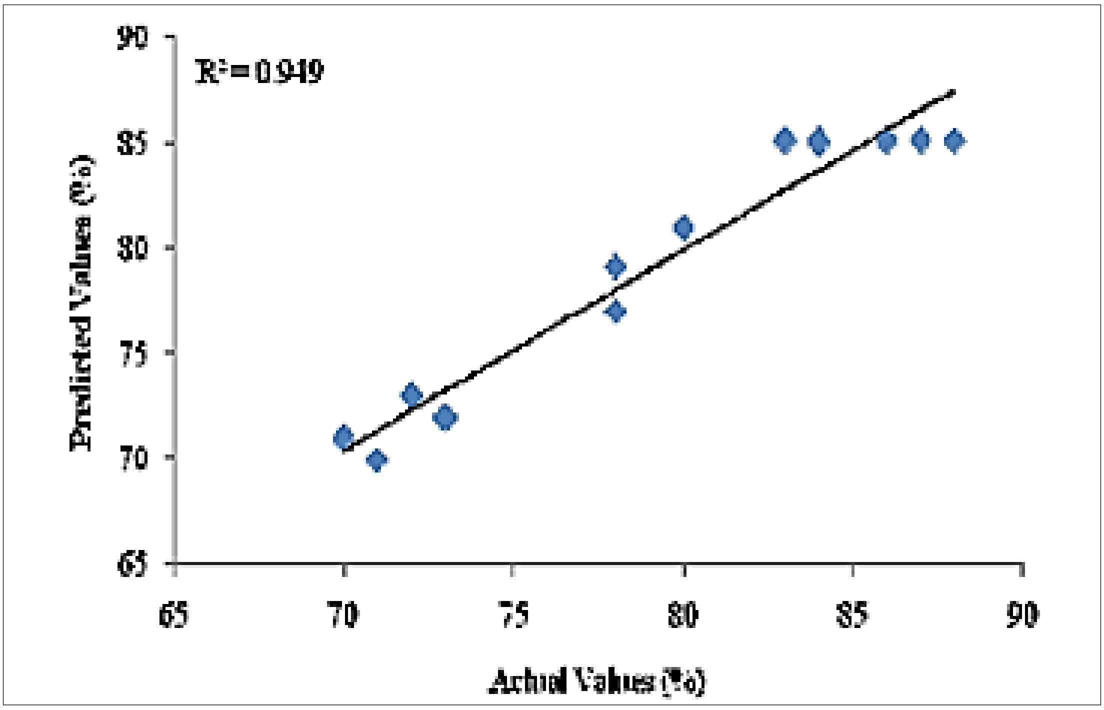
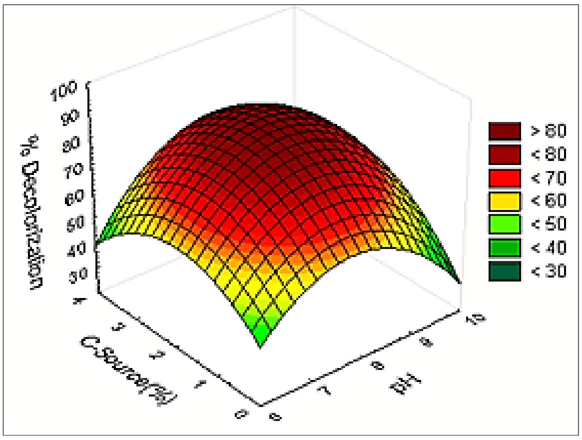
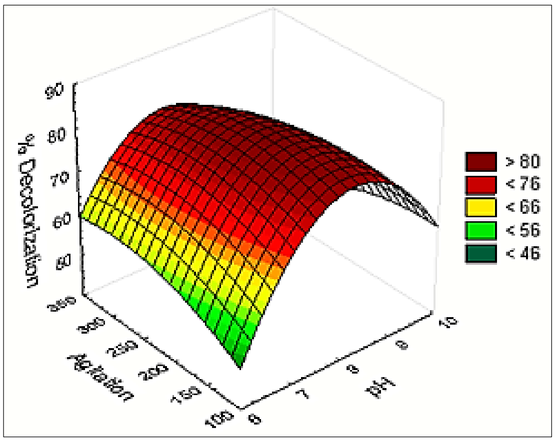


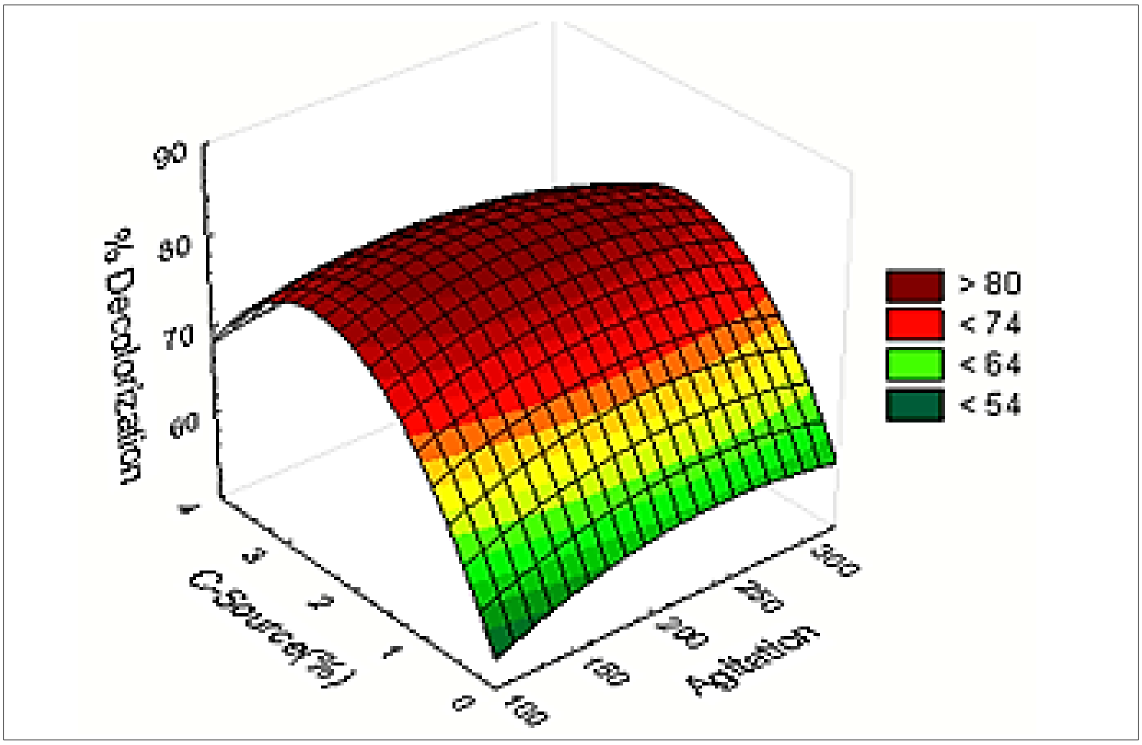
Fig 6: Regression line



A



B



C

Fig 7: A) Graphical represent comparing (A) agitation and pH **B)** carbon source and agitation **C)** carbon source and pH

Conclusion
In this study, I have isolated Three type of bacteria by enrichment method on the basis of morphology characteristics. During the course of screening of bacteria mixed culture

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