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Hydrocarbon Degradation using Fungal Isolate: Nutrients Optimized by Combined Grey Relational Analysis.

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Abstract
The potential biodegradation of hydrocarbon contaminants by microorganisms is dependent on the environmental factors and the nutrients available. In this study culture conditions like temperature, p^H, and nitrogen source were optimized by conventional one-factor at a time experimentation and the combination of other nutrients (nitrogen, phosphorus, magnesium, and sulfur) was optimized by using design of experiments (DOE) combined with grey relational analysis (GRA). Using GRA the multiple outputs were analyzed and normalized to get a grey grading (best combination).

Total petroleum hydrocarbons (TPH) of Oil sludge degradation was studied for a period of thirty days using a fungal strain isolated from the hydrocarbon contaminated soil. It has shown mixed results towards different oils. By the end of thirtieth day 87.1% of petroleum oil sludge was degraded by the fungal strain by producing 0.27 g/L of biomass, respectively.

Keywords - Biodegradation, fungus, grey relational analysis, total petroleum hydrocarbons, optimization.

I. INTRODUCTION

The petroleum constitutes (Hydrocarbons) are one of the main environmental pollutants. Their abundance and persistence in several polluted environmental areas have been reported [1]. The quality of the hydrocarbon content of the petroleum mixture influences the degradability of individual hydrocarbon components [2]. Oils which contain large quantities of heavy weight components may not undergo biodegradation easily as the molecules are too large and complex for the microbes to degrade [3].

In biodegradation process, microorganisms consume hydrocarbons as a carbon source. Carbon dioxide and water are released as waste products. Microbes which can utilize hydrocarbons are found naturally in the environment; however; they grow and develop after an oil spill occurs because additional carbon source (hydrocarbons) are available following a spill [3]. Biodegradation of petroleum hydrocarbon in the environment is found to be comparatively slow because it is influenced by a number of factors which include the microbial community which degrades the hydrocarbons, temperature and nutrient availability [4].

To enhance the bioremediation process, the microbial population present in the polluted environment is to be stimulated. Bio-stimulation involves the addition of nutrients to increase the rate of biodegradation process. In most shoreline ecosystems that have been highly contaminated with hydrocarbons, nutrients are likely the limiting factors in biodegradation of oil [5]. Nutrients addition in inappropriate quantity will not support the biodegradation process. So, addition of proper nutrients in appropriate quantity is very essential to promote or enhance the microbial growth. Recently nutrients optimization employing Taguchi method is reported as one of the predominant technique. Optimization using Taguchi method of orthogonal array (OA) design of experiments (DOE) involves the study of any given system by a set of independent variables (factors) over a specific region of interest (levels) [6]. This approach is adopted preferably because it is time saving and is a fully developed process. It provides systematic, sampling and efficient methodology with only a few well defined experimental sets for optimization of design parameters [7].

To optimize the nutrient components for a greater yield of fungal biomass and higher total petroleum hydrocarbon (TPH) degradation, preliminary experiments are to be conducted. The selection process of nutrients is based on fungal growth response towards the different nutrient concentration, where as the ranking determines which combination will yields the greater biomass. The numeric score for each nutrient is weighted by a factor, followed by summing all scores. The highest graded combination implies the best combination [8, 9]. Hence, the selection of the best nutrient combination in this traditional method is based on the response of fungal biomass and TPH concentration, which belongs to multiple attributes decision problems. Deng [10] had proposed Grey relational analysis in the Grey theory that was already proved to be a simple and accurate method for multiple attributes decision problems [11- 13], especially for those problems with very unique characteristic. Therefore, this study will utilize the Grey relational analysis to establish a complete and accurate evaluation model for selecting

appropriate nutrients combination. This methodology will yield higher fungal biomass with higher TPH degradation efficiency. [14]

This report uses the statistical design of the experiments combines with Grey relational analysis (GRA) to reduce the total number of attempts for optimizing the nutrients. It is expected that this statistical tool will derive the best nutrient combination for better growth of fungal biomass with higher TPH degradation efficiency. Statistical design of experiments determined the important effect of factors on a response as well as the effect of interaction among the factors [15]. Although statistical experimental design with a combination of GRA has largely been employed in the optimization process parameters in grinding, turning operations and welding works in mechanical industry [16, 17], it has been rarely applied to bioprocesses [15]. Thus application of Grey relational analysis forwards nutrient media optimization is employed in this study to enhance the biomass growth and TPH degradation using fungal strain isolated from petroleum hydrocarbon contaminated sites.

II. MATERIALS AND METHODS

The materials used in this study were Petroleum oil sludge collected from the HPCL (Vishakhapatnam, Andhra Pradesh). The other chemicals and nutrients were procured from Himedia laboratories and Merck chemicals.

2.1 Isolation of microorganism:

Microorganism used in the degradation study was isolated from the hydrocarbon contaminated water enriched with minimal salts and was inoculated with automobile work shop soil and left for two months at room temperature. Isolation of pure culture using serial dilution technique followed by inoculation on agar plate was adopted.

2.2 Media and growth conditions:

Microbial isolation experiments were performed in mineral salts medium (MSM) which contains (g 1^{-1}): 2.0g of Na_2HPO_4, 0.17g of K_2SO_4, 2.0g of $(NH_4)_2SO_4$, 0.53g of KH_2PO_4 and 0.10g of $MgSO_47H_2O$. The medium was autoclaved (subject to pressure sterilize at 121^oC) at 1.1 kg/cm^2 pressure for 15 min. Isolated pure cultures were tested for their ability to grow on solid MSM with 1.5% agar and 1% of the following pure hydrocarbons: n-Octane and n-Hexadecane [18-20]. Agar plates were incubated for 7 days at room temperature (28 - 30^oC) at a pH of 7.4 ± 0.2.

2.3 Characterization of Microorganism:

Based on morphology such as size, shape, color of spores formation and texture, the fungal strain was characterized. After 2-4 days of the growth of the fungi the spore bearing mycelia were carefully sectioned teased out and stained on a slide and then observed with a light microscope.

2.4 Gravimetric analysis:

Crude oil concentration was determined with a slight modification to the method applied by Rahman *et al.* Crude oil concentration was measured by mixing the oil samples with equal volume of n-Hexane to extract crude oil. The extracted crude oil was detected spectrophotometrically at 228 nm [21] using Jasco (V-530) UV/Visible spectrophotometer. The results were compared gravimetrically with the amount of residual crude oil (dry extract) separated from the culture flak by extraction (1:1 ratio) with n-hexane [1].

2.5 Biodegradation of TPH:

TPH degradation study using isolated fungal strains was determined by measuring the residual amount of crude oil present in culture after incubated. Experiments were carried out in 100 ml shaking flasks containing 50 ml optimized nutrient media in an incubator shaker at 120 rpm/min shaking speed at optimized temperature and p^H for 30 days. Petroleum oil sludge was added so that the initial amount of oil in the flask was set at 2.0 %.

2.6 Optimization of growth parameters:

Process parameters like temperature, p^H and nitrogen source were optimized by periodic experimentation and nutrients (nitrogen, phosphorus, magnesium and sulphate) are optimized by Gray based Taguchi method using Minitab 14 software.

A. The Taguchi Method:

The Taguchi method is a powerful tool in quality optimization for Bioprocesses. It uses a special design of orthogonal array (OA) to examine the quality characteristics through a minimal number of experiments. The experimental results based on the OA were converted into S/N ratios to evaluate the performance characteristics [6].

B. Grey Relational Analysis:

The grey relational analysis based on the grey system theory was used to solve the complicated interrelationships among the multiple responses effectively. In a grey system, all information was not available [15]. It is applicable in selection of microbial strains, optimization of nutrients, culture parameters, fermentation and bio degradation operations with multi-responses.

Data pre-processing is the primary stage in the grey analysis since the range and the unit in one data sequence may differ from the other. Data pre-processing is a means of transferring the original sequence to a comparable sequence. Depending on the characteristics of a data

sequence, there are various methodologies of data pre-processing available for this analysis [22].

The optimization of the process was performed in the following steps:

1. Normalizing the experimental results of fungal biomass and residual oil content for all experimental run.

2. Calculating the Grey Relational Coefficient (GRC).

3. Calculating the Grey Relational Grade (GRG) by averaging the GRCs.

4. Performing statistical analysis of variance (ANOVA) for the input parameters with the GRG and identifying the parameters significantly affecting the process.

5. Selecting the optimal levels of process parameters.

The indication of the better performances of fungal strain in TPH degradation study is biomass yield "higher the better" where as it is "lower the better" for residual oil content. In the analysis of Gary relation for "higher the better" response normalizing used Equation 1 and when the response is "lower the better", normalizing used Equation 2.

Response normalization for larger is better condition. $x_i^*(k)$

$$x_i^*(k) = \frac{x_i(k) - \min x_i(k)}{\max x_i(k) - \min x_i(k)} \quad (1)$$

Response normalization for smaller is better condition.

$$x_i(k) = \frac{\max x_i(k) - x_i(k)}{\max x_i(k) - \min x_i(k)} \quad (2)$$

Where $x_i^*(k)$ and $x_i(k)$ the normalized data and observed data, respectively, from i^{th} experiment using k^{th} response. The smallest and the largest value of $x_i(k)$ for the k^{th} response are min $x_i(k)$ and max $x_i(k)$, respectively. After pre-processing the data, the Gray relation coefficient $\xi_i(k)$ for the k^{th} response characteristics in the i^{th} experiment can be expressed as follows.

$$\xi_i(k) = \frac{\Delta \min + \xi \Delta \max}{\Delta_i(k) + \xi \Delta \max} \quad (3)$$

Where $\Delta i(k)$ is the k^{th} value in Δ_i different data series. Δ_{max} and Δ_{min} are the global maximum and minimum values in the different series, respectively. The distinguished coefficient ξ lies between 0 and 1, which is to expand or compress the range of GRC. The distinguishing coefficient can be selected by decision maker's judgment, and different distinguishing coefficients usually provided different results GRG. The most preferred value of $\xi = 0.5$.

After calculating GRC, for n number of responses, the GRG (γ) can be calculated using Equation 4.

$$\gamma = \frac{1}{n} \sum_{i=1}^{n} \xi_i(k) \quad (4)$$

The magnitude of γ reflects the overall degree of standardized deviation of the i^{th} original data series from the reference data series. In general, a scale item with a high value of γ indicates that the respondents, as a whole, have a higher degree of favorable consensus on the particular item.

III. RESULTS AND DISCUSSION

3.1 Isolation of microorganism:

A solution of 500 ml water which contains 5.25 grams of minimal salts and 1% of engine oil was inoculated with 5gm of soil and left alone for 2 months in a beaker. At the end of 2 months some fungal growth was observed. A fungal mycelia growth was observed over the hydrocarbon contaminated water. 1 ml of fungal contaminated water from the white zone (fig 1(a)) was then taken and serially diluted at the order of 10^{-2} was cultured on a solid MSM containing 1.5% agar and 1% of n-Octane as a carbon source and further tested on n-Hexadecane. Culture plates were incubated for 10 days at room temperature (28 - 30ºC) at a pH of 7.4 ± 0.2. After 10 days fungal colonies were observed on the petri dishes which indicate the efficient utilization of hydrocarbons (n-Octane and n-Hexadecane) as a sole carbon source by the microorganism (fig 1(b)).

Isolated pure culture was tested for its ability to grow on hydrocarbons, for that isolated fungal culture is grown on petri dishes containing solid MSM

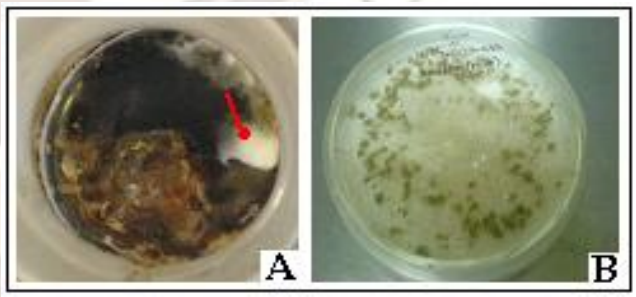

Figure 1: (a) Fungal growth on MSM flask inoculated with automobile work shop soil containing hydrocarbons as a carbon source. **(b)** Fungal colony growth on MSM containing n-Octane as a sole carbon source.

3.2 Characterization of Microorganism:

Fungal strain was characterized to identify the family to which it belongs. Characterization is based on the morphology of colony and conidiophores were adopted.

Colonies were light yellowish to brown; reverse pink colored. Conidiophores were colorless. Vesicles were elongate with metulae and phialides, covering most of the surface. Conidia were globose to subglobose, smooth to echinulate, 4-5µm in diameter. These characteristics helps to conclude that the isolated fungal strain belonged to the genus Aspergillus and the species was *Aspergillus versicolor*.

3.3 Optimization of growth parameters:

A. Temperature Optimization:

Temperature has a significant effect on the rate of microbial growth. [6]. Temperature influences petroleum hydrocarbon biodegradation by its effect on the physical nature and chemical composition of the oil, rate of hydrocarbon metabolism by microorganisms, and composition of the microbial community [4, 23].

The temperatures considered for optimization were 20ºC, 25ºC, 30ºC, 35ºC and 40ºC. The culture is prepared in 5 different flasks, each maintained at different temperature. In this study p^H of 7.4 ± 0.2 was maintained, since isolation of fungal strain was conducted at this p^H. Experiment was carried out in 100 ml conical flasks containing 50 ml MSM media with 0.5gms of petroleum oil sludge. The flasks were kept in shaker incubator at 120 rpm, for 15 days. At the end of the experiment the yield of fungal biomass in conical flask incubated at 30ºC temperatures was measured as 1.43 g/L which produced highest biomass and the flask incubated at 20ºC and 40ºC temperatures produced 0.026 and 0.025 g/L, which showed the least biomass yield (fig 2). Thus 30ºC temperatures was the optimum temperature for the growth of *Aspergillus versicolor*.

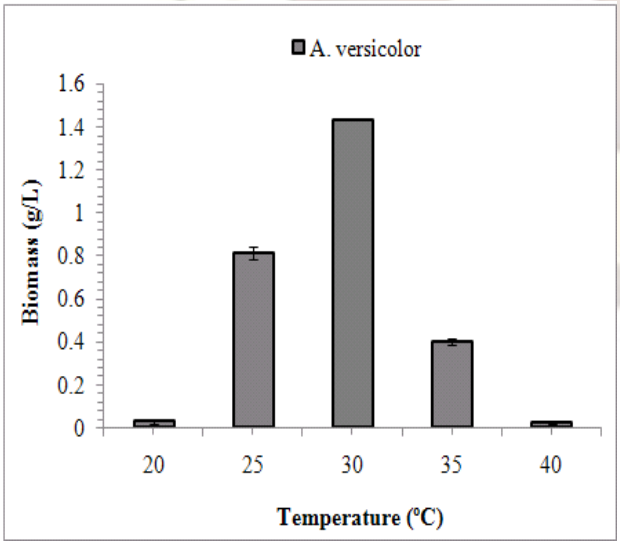

Figure 2: Biomass yield at different incubation temperatures.

B. p^H Optimization:

The p^H considered for optimization ranges from 6 to 9. The culture was prepared in 4 different flasks; each maintained at a different p^H. The concentration of the broth and the carbon source was the same as in temperature optimization. The p^H was adjusted by adding 0.1N solution of NaOH and HCl. The flasks were sterilized, and kept in shaker incubator chamber at 120 rpm for 15 days and the temperature maintained at 30ºC.

At different p^H 6, 7, 8 and 9 the *Aspergillus versicolor* biomass yield demonstrated 0.58, 1.67, 1.48 and 0.29 g/L respectively. p^H 7-8 was considered as optimum condition for growth of *Aspergillus versicolor*. Fig.3 denotes the fungal biomass growth at different p^H.

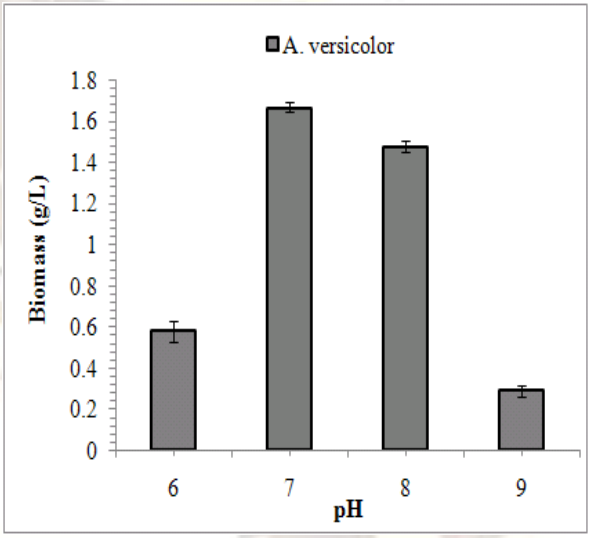

Figure 3: Biomass yield at different p^H.

C. Nutrients Optimization:

Nutrients such as nitrogen, phosphorus, magnesium, and sulfur play a much more critical role in limiting the rate of hydrocarbon biodegradation. The ratio of available carbon to nitrogen in the bioremediation system has been identified as the significant parameter affecting degradation rate [24]. Recent studies found that the hydrocarbon biodegradation rate depends on the nutrient concentrations in the contaminated site which provides important guidance for nutrient applications [2]. Minor trace elements are usually present in sufficient amounts in the environment [4]. So, optimization of macro nutrients (nitrogen, phosphorus, magnesium, and sulfur) to promote fungal biomass growth is very essential.

For optimizing the nitrogen source, peptone, ammonium sulfate, ammonium chloride, and urea were used at a concentration of 0.5%. The culture was prepared in 4 different flasks and maintained at an optimized temperature 30ºC and p^H 7.0. The concentration of the broth and the carbon source was the same as in

temperature and p^H optimization. The yield of *Aspergillus versicolor* biomass at different nitrogen sources such as peptone, ammonium chloride, ammonium sulphate and urea were obtained as 3.24, 0.92, 1.62 and 2.19 g/L respectively. Thus biomass yield was maximum with peptone and best with Urea. Fig.4 denotes the fungal biomass growth at different p^H.

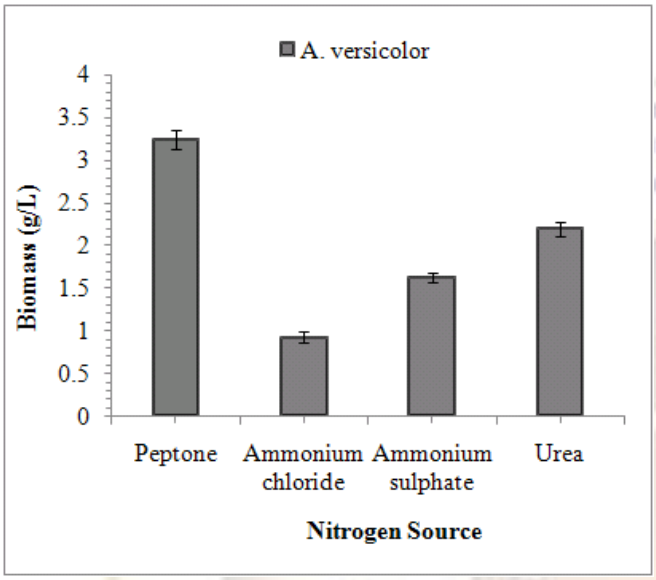

Figure 4: Biomass yield at different nitrogen sources.

D. Optimization by Taguchi Method:

The conventional one factor at a time approach of optimization is not only incapable in its interactions but often time consuming. The method becomes impractical when a large number of nutrients in the culture medium have to be considered since too many combinations have to be considered to optimize the growth medium composition. The orthogonal matrix method can be used to investigate the relationships between variables of nutrient medium components and optimize their concentrations for fungal cell growth and percentage degradation of TPH's. The orthogonal matrix method, as a result of the suitable design of factors, can give effective responses [25]. This implied that the selected conditions were the most suitable in practice. In fact, they have been successfully applied in bioprocess industry for the improvement of culture media for a better production of primary and secondary metabolites using fermentation process [6, 15].

The control factors showed in Table I which include four different levels of sulphates, phosphates, nitrites and magnesium were used as nutrient salt sources in the TPH degradation study.

TABLE I: Factors and levels in the TPH degradation study

Factors	Optimization parameters	Unit	Level 1	Level 2	Level 3	Level 4
A	Sulphates	g/L	0.4	0.6	0.8	1.0
B	Phosphates	g/L	0.4	0.6	0.8	1.0
C	Nitrites	g/L	0.4	1.5	2.0	2.5
D	Magnesium	g/L	0.1	0.2	0.3	0.4

TABLE II: L16 (4^4) Orthogonal array of the experimental design and its result's, fungal biomass and residual TPH in culture flask and their respective s/n ratios.

Exp. No.	Factors				Biomass concentration (g/L)	Residual oil (%)
	A	B	C	D		
1	0.4	0.4	1.0	0.1	1.425	22.61
2	0.4	0.6	1.5	0.2	1.250	14.45
3	0.4	0.8	2.0	0.3	2.285	14.58
4	0.4	1.0	2.5	0.4	2.390	15.52
5	0.6	0.4	1.5	0.3	1.700	14.09
6	0.6	0.6	1.0	0.4	2.225	13.99
7	0.6	0.8	2.5	0.1	1.940	15.73
8	0.6	1.0	2.0	0.2	2.545	12.71
9	0.8	0.4	2.0	0.4	2.025	19.10
10	0.8	0.6	2.5	0.3	2.275	13.75
11	0.8	0.8	1.0	0.2	2.425	12.27
12	0.8	1.0	1.5	0.1	2.300	16.51
13	1.0	0.4	2.5	0.2	2.195	18.66
14	1.0	0.6	2.0	0.1	2.025	17.10
15	1.0	0.8	1.5	0.4	1.980	13.79
16	1.0	1.0	1.0	0.3	2.335	13.14

Taguchi method designed an orthogonal array of sixteen experiments (L_{16}) arranging the four input factors and corresponding levels in crisscross way. These sixteen experiments were performed under culture conditions which optimized earlier by one factor at a time approach, was with temperature 30^0C, p^H 7.0, nitrogen source as urea, carbon source as petroleum oil sludge (0.5 %) the

flasks were kept under shaking at 120 RPM for a time period of 15 days.

Aspergillus versicolor biomass and residual oil were taken for consideration and results of L_{16} experiments are given in Table II. The results of L_{16} experiments S/N ratios are given in Table III.

TABLE III: S/N ratio results of fungal biomass and residual TPH in L_{16} experiments.

Exp. No.	S/N ratio of biomass	S/N ration of residual oil
1	3.08	-27.09
2	1.94	-23.20
3	7.18	-23.28
4	7.57	-23.82
5	4.61	-22.98
6	6.95	-22.92
7	5.76	-23.93
8	8.11	-22.08
9	6.13	-25.62
10	7.14	-22.77
11	7.69	-21.78
12	7.23	-24.35
13	6.83	-25.42
14	6.13	-24.66
15	5.93	-22.79
16	7.37	-22.37

From the results of L_{16} (4^4) orthogonal arrays (Table II), two procedures were conducted to obtain GRG (γ) as shown in Table V. The response graph is based on the average values of the grey relational coefficient the optimal conditions were selected. The normalized raw data of Aspergillus versicolor biomass (Table II) and their calculated values (Table IV) were represented employing larger-the-better and normalized function respectively (Eq.1). The normalization of residual oil was evaluated using smaller-the-better normalized function of GRA (Eq.2). Grey relational coefficients ($\xi_i(k)$) and their grey grades were obtained through Eq.3 and Eq.4 to evaluate the multiple performance characteristics, respectively.

TABLE IV: Grey relational analysis response table

Exp. No	Biomass	Oil	Normalized Values		G R Coefficient	
			Biomass	Oil	Σ Biomass	Σ
1	1.425	22.61**	0.8649	1.0000	0.37	0.33
2	1.250*	14.45	1.0000	0.2109	0.33	0.70
3	2.285	14.58	0.2008	0.2240	0.71	0.69
4	2.390	15.52	0.1197	0.3145	0.81	0.61
5	1.700	14.09	0.6525	0.1761	0.43	0.74
6	2.225	13.99	0.2471	0.1660	0.67	0.75
7	1.940	15.73	0.4672	0.3344	0.52	0.60
8	2.545**	12.72	0.0000	0.0425	1.00	0.92
9	2.025	19.10	0.4015	0.6605	0.56	0.43
10	2.275	13.75	0.2085	0.1435	0.71	0.78
11	2.425	12.27*	0.0927	0.0000	0.84	1.00
12	2.300	16.51	0.1892	0.4101	0.73	0.55
13	2.195	18.66	0.2703	0.6180	0.65	0.45
14	2.025	17.10	0.4015	0.4673	0.56	0.52
15	1.980	13.79	0.4363	0.1474	0.53	0.77
16	2.335	13.14	0.1622	0.0846	0.75	0.86

***** = Lower value; ****** = Higher value.

TABLE V: GRG values and Grey Ranking

Experiment	GRG γ	S/N Ratio	Mean	Gray Ranking
1	0.3498	-9.119	0.350	16
2	0.5183	-5.713	0.518	14
3	0.7021	-3.073	0.702	7
4	0.7104	-2.975	0.710	5
5	0.5867	-4.627	0.587	10
6	0.7100	-2.975	0.710	6
7	0.5581	-5.067	0.558	11
8	0.9609	-0.346	0.961	1
9	0.4927	-6.143	0.493	15
10	0.7414	-2.604	0.741	4
11	0.9218	-0.705	0.922	2
12	0.6374	-3.917	0.637	9
13	0.5482	-5.224	0.548	12
14	0.5358	-5.417	0.536	13
15	0.6532	-3.702	0.653	8
16	0.8052	-1.884	0.805	3

GRG is the overall representative of both the responses. The multi response optimization problem was transformed into a single response by using this approach. S/N ratio and mean values obtained from the GRG values were shown in Table V. The experimental trial No 8 had a higher grey relational grade, 0.9609, than other experimental trials and the experimental trial No 1 had a lower grey relational grade, 0.3498. Normally, the larger the grey relational grade, the closer the product quality to the ideal value. The optimum conditions for TPH degradation by Aspergillus versicolor were sulphates 0.6 g/L (A_2), phosphates 1.0 g/L (B_4), Nitrites 2.0 g/L (C_3) and magnesium 0.2 g/L (D_2).

Using GRG value, ANOVA was designed for identifying the significant factor. The results of ANOVA were presented in Table VI. Predominance of phosphates 1.0 g/L on maximum *Aspergillus versicolor* biomass growth and higher percentage of TPH degradation is clearly represented. Subsequently magnesium 0.2 g/L, sulphates 0.6 g/L and Nitrites 2.0 g/L showed their responses in decreasing order. The mean of these response graph based on GRG with four variables and levels illustrated in figure 5. This study using Grey based Taguchi enables to understand the influence of four different nutrients and their concentration on the growth of fungal biomass and TPH degradation efficiency of *Aspergillus versicolor*.

TABLE VI: Analysis of variance for means

Means

Source	DOF	Seq SS	Adj MS	F	P	% Contribution	Rank
A	3	0.0472	0.0157	2.20	0.266	12.40	3
B	3	0.1789	0.0596	8.35	0.057	46.95	1
C	3	0.0216	0.0072	1.01	0.497	5.68	4
D	3	0.1118	0.0372	5.22	0.104	29.35	2
Residual Error	3	0.0214	0.0071			5.62	
Total	15	0.3811				100	

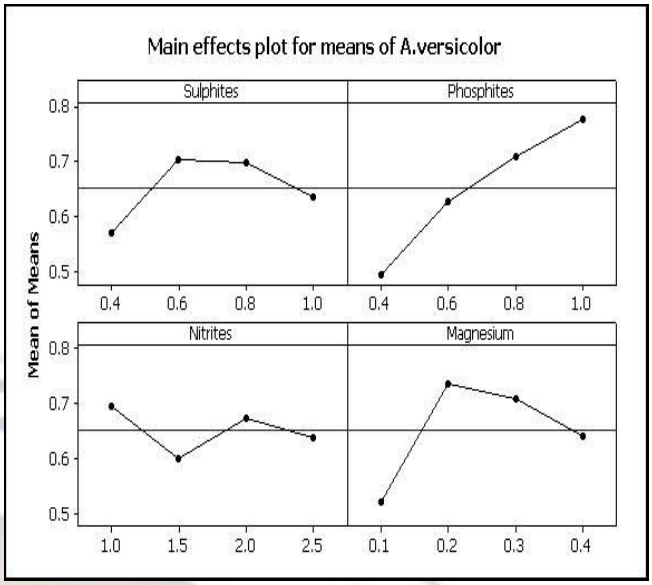

Figure 5: The response graph of means based on grey relational grades.

D. Degradation of TPH:

Maximum uptaking of TPH from oil sludge was elucidated using by the *Aspergillus versicolor*. Three sets of experiment were conducted to study the growth of fungal biomass and TPH degradation. Each set of flask were examined for the *Aspergillus versicolor* growth and oil sludge degradation at a regular interval of 10 days.

At the end of thirty days fungal biomass exhibited maximum growth (27.1 g/L) in the flask containing oil sludge as a carbon source. This figure shows decreasing trend with increase in Aspergillus versicolor life cycle. However their abilities to degrade a specific hydrocarbon as a source of energy and/or biomass may differ. The chemical composition of a crude oil may also be a factor in determining the type of fungi, which may grow on it [26, 27].

By the end of thirtieth day culture flask containing oil sludge as a carbon source has shown 87.1%, with a final biomass concentration of 2.71 g/L. Significant biomass growth and decrease in oil concentration suggested that oil sludge is easily degraded by the *A. versicolor.* Figure 6 shows the removal of oil sludge from the surface of the nutrient media and growth of fungal mycelium. Fungal biomass exhibits a rapid growth by utilizing the oil sludge as a carbon source. Fungus produced mycelia and immobilized the oil droplets. Decrease in oil droplet size was clearly visible which indicated the degradation of oil within the mycelium.

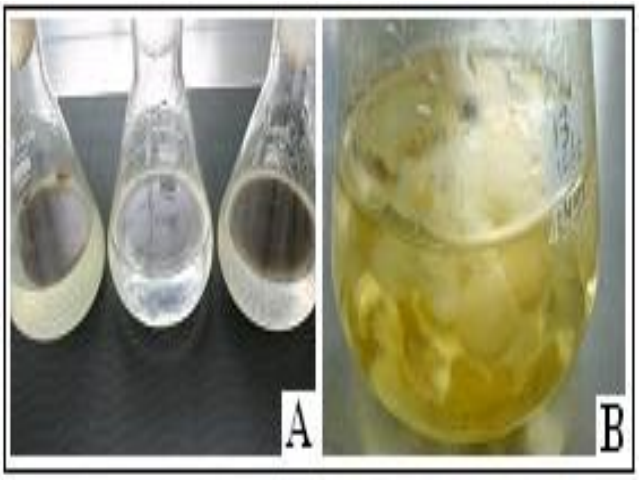

Figure 6: (a) Culture flasks containing oil sludge. (b) Fungal mycelia growth on oil sludge.

Degradation of petroleum oil sludge by *Aspergillus versicolor* in a time period of thirty days was shown in the Figure 7. Experimental results show that there is a decrease in the oil sludge concentration with the increase in the quantity of fungal biomass. Maximum growth of *Aspergillus versicolor* biomass was found till twenty days and later we have observed a declination stage in the *Aspergillus versicolor* growth cycle which results in the 82.4% degradation of oil sludge for first twenty days and later 4.7 % degradation till thirtieth day of study. It may be due to the limiting of nutrients in the culture medium. Nutrients play a key role in the metabolic activates, such as production of many intracellular enzymes which metabolizes the hydrocarbons and helps in the biomass production and in cell growth [5]. It might be the reason for the decrease in the growth of *A. versicolor* biomass.

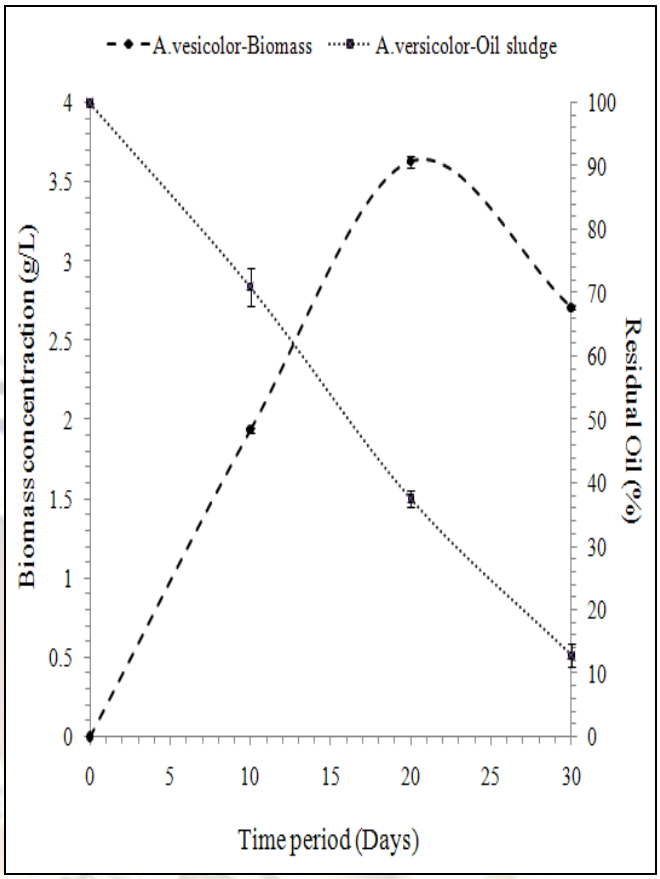

Figure 7: Growth of fungal biomass and petroleum oil sludge degradation.

IV. CONCLUSION

Grey-based Taguchi method could effectively improve and solve nutrients optimization problems faced in biodegradation of organic contaminant process with multiple performance characteristics. Higher degradation efficiency can be achieved on the basis of these optimal culture conditions. Fungal strain isolated from hydrocarbon contaminated soil could degrade 87.1% of oil sludge in a period of 30 days. These results were obtained at optimum nutrient conditions such as sulphates 0.6 g/l, phosphates 1.0 g/l, nitrites 2.0 g/l and magnesium 0.2 g/l which are optimized by using Grey based Taguchi method. Other optimized parameters such as temperature and p^H were considered as 30^oC and 7.0 respectively. This study proved that application of statistical model like Grey-based Taguchi method in biological process help in transforming the multiple responses into a single response.

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