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OPTIMIZATION OF PARAMETERS FOR DENITRIFICATION OF WASTEWATER USING FLUIDIZED BED BIOREACTOR BY TAGUCHI METHOD

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Abstract- The objective of this study is to investigate the optimal parameter levels for removal of nitrates from wastewater using *Pseudomonas stutzeri* in a fluidized bed bioreactor by Taguchi method and validating the predicted values. In this study the influence of airflow rate, temperature, carbon source, PP beads (Poly Propylene) and pH at different levels was investigated was made using Taguchi's $L_{16}(4)^5$ orthogonal array technique. The nitrate removal efficiency after optimization is more than 96% with airflow rate at level 2 (A_2), temperature at level 3(B_3), carbon source at level 4(C_4), PP beads at level 2(D_2) and pH at level 2(E_2). The analysis of variance (ANOVA) test is used to determine the percent contribution of each parameter and found that airflow rate contribution is the highest and temperature provides the least contribution.

Keywords - Wastewater, Biological denitrification, Fluidized bed bioreactor, *Pseudomonas stutzeri*, Taguchi method, ANOVA

INTRODUCTION

Nitrate is a major pollutant present in effluent wastewater from nitrogenous fertilizer, metal ores, explosives, paper mills, pulp mills, explosive industries and also from municipal waste. Nitrate is harmful to both mankind and animal and also to the environment. Nitrates causes cancer, blue-bay syndrome, hypertension and thyroid hypertrophy. Hence drinking water regulations are required to strictly implement in order to limit human risks and environmental pollution. The United States Environmental Protection Agency (USEPA) has set maximum contaminant level goal (MCLG) of 10 mg NO_3-N and 1.0 mg NO_2-N/lit, the World Health Organization and European Economic Community have set standards of 11.3 an0.03 mg NO_2/lit. Water above these limits requires denitrification. There are different methods, which have been developed for removing nitrogen like ion exchange [1], reverse osmosis [2], electrodialysis [3], chemical and catalytic denitrification [4]. Each of them has its own advantages and disadvantages. Among these conventional means for NO_3-N removal from drinking water include ion exchange and variety of membrane technologies, such as reverse osmosis.

These processes have been proven effective in NO_3-N removal [5]. However, disadvantages, including poor selectivity for NO_3-N, concentrated waste disposal issues, cost, and susceptibility to fouling have fed the search for alternative NO_3-N removal of technologies. Biological method is found to be the most commonly used and effective method. In this process microorganisms first reduce nitrates to nitrites and then produce nitric oxide, nitrous oxide and nitrogen gas. The pathway for nitrate reduction is:

$$NO_3 \rightarrow NO_2 \rightarrow NO \rightarrow N_2O \rightarrow N_2$$

Several studies has been carried on denitrification [6-14] but work reported on biological denitrification of waste water using a fluidized bed bioreactor is very little using *Pseudomonas stutzeri*.

A number of investigations are reported in the literature on the nitrate removal from wastewater. An extensive review has been carried out by Mousavi et al. 2007 [15] to find the optimum values of ferrous biooxidation rate by immobilization of native *Sulfobacillus sp.* on the surface of LDPE particles in a packed bed bioreactor using taguchi method for five control factors.

Madaeni et al., 2006 [16], have reported on overview of the application of taguchi method in the flux optimization of wastewater treatment. Uludag-Demirer Sibel et al., 2008 [17] has also made a review on removal of nitrate and phosphate, and on experimental design by Taguchi' $L_9(3^4)$ orthogonal array technique. Shrimali et al., 2001 [18], reported on new methods like ion exchange, reverse osmosis and electro-dialysis for nitrate removal from water and that the utility of these processes has been limited due to their expensive operation and subsequent disposal problem of the generated nitrate waste brine.

Abbas Rezaee et al., 2008 [19], investigation was directed upon the feasibility of a biological denitrification process using immobilized *Pseudomonas stutzeri* with microbial cellulose from *Acetobacter xylinum* as the support material for the immobilization of the bacterium to remove the nitrates. Yuguang Li et al., 2007 [20] reported optimization process parameter of petroleum bio desulfurization by Taguchi methodology .

MATERIALS AND METHODS

Bacterial strain and Cultivation

A pure culture of *Pseudomonas stutzeri*, a denitrifying bacterium was obtained which is capable of utilizing nitrates as the energy source. The culture was preserved in refrigerator at a temperature of 4 ^{0}C by periodic sub culturing on nutrient agar. The bacterium was sub cultured once in a month and grown in the composition containing (per liter) 10 g of peptone, 10 g of beef extract, 5 g of Nacl and 20 g of agar-agar for the slant preparation.

Preparation of Synthetic wastewater and Cell immobilization

The experimental work was carried out in a Fluidized bed bio-reactor with attached growth process for the removal of nitrate from synthetic wastewater. Polypropylene beads of density 600 kg/m^3 and of 1.96 mm in diameter are used as the supporting media for the growth of microorganism. The bacterium from the slants was inoculated into liquid broth containing nitrate concentration of 30 mg/L and was prepared by mixing: 48.9 mg of KNO_3, 85 mg of CH_3OH, 6 mg of $MgSO_4.7H_2O$, 0.2 mg of $FeCl_3.7H_2O$, 430 mg of Na_2HPO_4 and 320 mg of $Na_2H_2PO_4$. The composition gives the initial nitrate concentration of 30 mg/L, to increase or decrease the nitrate composition varies the amount of potassium nitrate proportionately. The experiment was conducted initially for 150, thereafter 180 and 200 ppm.

Experimental Set-up of the fluidized bed bioreactor

The Fluidized bed bio-reactor consists of a glass column of 0.5m height, 93 mm of Internal Diameter and 100mm of Outer Diameter with a capacity of 3.4 liters. The setup was provided with a glass jacket of 118 mm ID and 122 mm OD, to maintain the temperature of the reactor system at the set point and also provision was made for the supply of air/N_2/O_2 based on the requirement. A gas sparger was located at the base of column for uniform distribution of gas [21].

Analytical methods

Samples of nitrate were collected for every 1 hour, filtered and were used for the analysis of final nitrate concentration with UV- Visible Spectrophotometer according to standard methods [22].

ANALYSIS OF EXPERIMENTAL RESULTS BASED ON TAGUCHI METHOD

The Taguchi method, a powerful tool for parameter design of the performance characteristics was used to determine the optimal parameters for denitrification of wastewater using FBBR. In this study the behavior of five control factors, A, B, C, D, and E with four levels was optimized as shown in Table (1).

The array chosen was the $L_{16}(4)^5$ [28], which has 16 rows corresponding to the number of experiments with 5 input factors at four levels as shown in Table 2. The plan of experiments is as follows (Table 2): the first column was assigned to number of trials , the second column to Air flow rate (A), the third column to Temperature (B), the fourth column to Carbon Source (C), the fifth column to Poly Propylene beads (D) and the sixth column assigned to pH (E). The experiments were conducted for each combination of factors as per selected orthogonal array.

Table-1-Controllable Parameters and Levels for optimization:

Parameters	Level1	Level2	Level3	Level4
A: Airflow rates (lpm)	2	2.5	3	3.5
B: Temperatures (^{0} C)	20	25	30	35
C: Carbon Source (mg/L)	70	75	80	85
D: PP beads (gm)	10	15	20	25
E: pH	6	7	8	9

The experimental values of Nitrates removal (Y) in ppm after 12 hours for 16 runs (n) according to Taguchi method are transformed into a signal-to-noise (S/N) ratio as shown in Table (2).

Signal-to- noise ratio

The signal to noise ratio (S/N ratio) was used to measure the sensitivity of the quality characteristic being investigated in a controlled manner. In Taguchi method, the term "signal" represents the desirable effect (mean) for the output characteristic and the term "noise" represents the undesirable effect (signal disturbance, S.D) for the output characteristic which influence the outcome due to external factors namely noise factors.

The S/N ratio can be calculated as a logarithmic transformation of the loss function as shown below.

$$\frac{S}{N} ratio = -10\log_{10}\left(MSD\right) \qquad (1)$$

Where 'MSD' is Mean Square Deviation

The aim of any experiment is always to determine the highest possible S/N ratio for the result. There are three possible categories of the quality characteristics, they are: (1) smaller is better, (2) nominal is better and (3) bigger is better. To obtain maximum removal of nitrates, the 'bigger is better' quality characteristic was chosen. The mean square deviation (M.S.D.) for 'bigger is better' quality characteristic can be expressed as:

$$MSD = \frac{1}{n}\sum_{i=1}^{n} 1/Y_i^2 \qquad (2)$$

Where 'n' number of repetitions

'Y_i' the observed data obtained from present experimental work.

Table 2 shows the experimental results for removal of nitrates and the corresponding S/N ratio using results of the experiments from Equations (1) and (2). Since the experimental design is orthogonal, it is then possible to separate out the effect of each parameter at different levels.

Analysis of Variance

The analysis of variance (ANOVA) is a common statistical technique to determine the percent contribution of each parameter for

$$SS_T = \sum_{i=1}^{n} SN_i^2 - CF \; ; \qquad (3)$$

$$CF = \left(\sum_{i=1}^{n} SN_i\right)^2 \Big/ n \qquad (4)$$

$$SS_A = \frac{A_1^2}{N_{A_1}} + \frac{A_2^2}{N_{A_2}} + \frac{A_3^2}{N_{A_3}} + \frac{A_4^2}{N_{A_4}} - CF \qquad (5)$$

f_A= levels of factor A-1; (6)

$$V_A = \frac{SS_A}{f_A} \qquad (7)$$

$$P_A = \frac{SS_A}{SS_T} \qquad (8)$$

Where
SS_T is the total sum of squares, n is the number of experiments in the orthogonal array,
SN is S/N ratio, CF is the correction factor, SS_A is sum of squares for factor A, A_1 is observed value at level 1 of parameter A, N_{A1} is number of experiments of parameter A at level 1, f_A is degree of freedom of parameter A, V_A is variance and P_A is percentage contribution of parameter A.
By applying the above equations, the sum of squares, variance and percentage contribution of each parameter was calculated and they were organized in a standard tabular format known as ANOVA table (Table 4).

RESULTS AND DISCUSSION

The current study applies the Taguchi method to determine the optimum parameters and the individual contribution for the denitrification of waste water. In this study, for the design of the experiment with a matrix of 5 parameters with 4 levels, there will be as many as 1024 runs of testing using the conventional method. The testing of only 16 runs with the Taguchi method greatly reduces the number of tests and increases the efficiency.
The mean values of S/N ratios for nitrate removal at 200 ppm are given in Table 3 keeping the objective as “Bigger is better”. In order to quantify influence of each level of parameters, Mean of S/N ratio for parameter A at levels 1, 2, 3, and 4 were computed by averaging S/N ratios for experiments 1-4, 5-8, 9-12 and 13-16 respectively. Mean of S/N ratio for each level of other parameters were calculated in a similar manner. The mean S/N ratio for each level of the parameters is summarized and called the S/N response table for removal of nitrates (Table 3). “Figures (1), (2), (3), (4) and (5)” show the S/N response graph for removal of nitrates at 200 ppm. As shown in Equation (1) and (2), the greater is the S/N ratio, the smaller is the variance of nitrates removal around the desired (the-bigger-the-better) value. However, the relative importance amongst the parameters for nitrates removal still needs to be known so that optimal combinations of the parameter levels can be determined more accurately.

able-2-Nitrates removal (Y_{avg}) in ppm with S/N ratios after 12h:

Orthogonal array for $L_{16}(4)^5$ Taguchi design 200ppm

$L_{16}(4)^5$	A	B	C	D	E	Y_{avg}	S/N ratio
1	1	1	1	1	1	171	44.655
2	1	2	2	2	2	181	45.143
3	1	3	3	3	3	175	44.853
4	1	4	4	4	4	172	44.703
5	2	1	2	3	4	176	44.908
6	2	2	1	4	3	178	45.978
7	2	3	4	1	2	190	45.574
8	2	4	3	2	1	189	45.501
9	3	1	3	4	2	180	45.073
10	3	2	4	3	1	186	45.386
11	3	3	1	2	4	184	45.264
12	3	4	2	1	3	182	45.172
13	4	1	4	2	3	179	45.05
14	4	2	3	1	4	174	44.809
15	4	3	2	4	1	177	44.955
16	4	4	1	3	2	173	44.753

Table-3-S/N ratio response table at 200 ppm after 12h:

Parameters	Level 1	Level 2	Level 3	Level 4
A: Airflow rate (lpm)	44.839	45.24	45.224	44.892
B: Temperature (°C)	44.922	45.079	45.162	45.032
C: Carbon Source (mg/L)	44.913	45.045	45.059	45.178
D: PP beads (gm)	45.053	45.24	44.975	44.927
E: pH	45.125	45.136	45.014	44.921

Table-4-ANOVA Table for 200 ppm:

Parameters	Sum of Sqrs (S)	Variance (V)	Percent (P)
A: Airflow rate (lpm)	0.542	0.18	46.93
B: Temperature (°C)	0.12	0.04	10.398
C: Caron Source (mg/L)	0.139	0.046	12.088
D: PP beads (gm)	0.226	0.075	19.547
E: pH	0.123	0.041	10.648

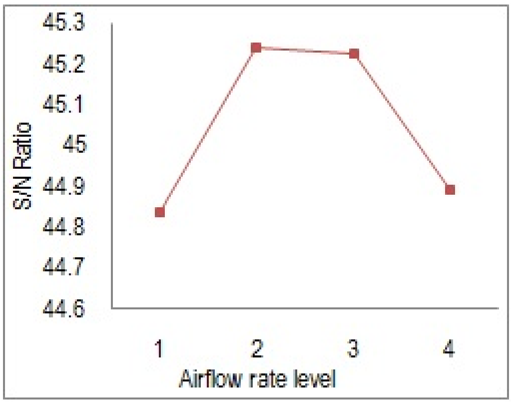

Fig. 1- Effect of Airflow rate on S/N ratio

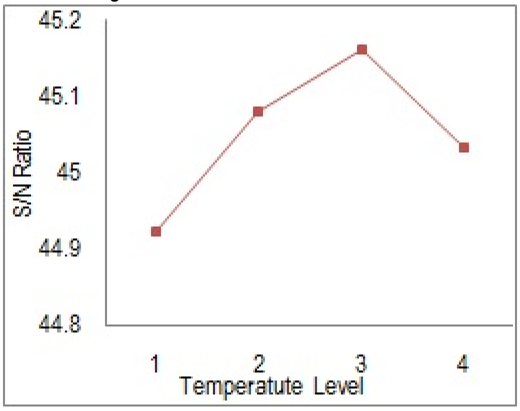

Fig. 2- Effect of Temperature on S/N ratio

It is clear from “Figures (1), (2), (3), (4) and (5)” that Nitrate removal is maximum at the 2nd level of parameter A, 3rd level of parameter B, 4th level of parameter C , 2nd level of parameter D and 2nd level of parameter E. The S/N ratio analysis suggests the same levels of the parameters (A_2 B_3 C_4 D_2 and E_2) as the best levels for maximum removal of nitrates.

The knowledge of the contribution of individual parameters is critically important for the control of the final response. In order to study the significance of the parameters in effecting the quality characteristic of interest i.e. nitrates removal, ANOVA was performed. Table (4) shows the results of Analysis of Variance (ANOVA) for nitrates removal. The contribution for these parameters was air flow rate (46.93%), temperature (10.398%), carbon source (12.088%), PP beads (19.547%) and pH (10.648%). Thus, based on the S/N ratio and ANOVA analyses, the optimal combination of parameters and their levels for achieving maximum nitrates removal was A_2 B_3 C_4 D_2 and E_2.

For analysis of the result and optimization of conditions for setting the control parameters QUALITEK-4 software was used. QUALITEK-4(QT4) version 4.75 is the windows version software for Automatic Design and Analysis of Taguchi Experiments. According to the Taguchi method, airflow rate has the highest contribution and temperature provides the least contribution for maximum nitrates removal from wastewater as shown in “Figure (6)”. The best setting for control parameters is Airflow rate = 2.5 lpm, Temperature = 30^0 C, Carbon source = 85 mg/lit, PP beads=15 gm/lit and pH=7.

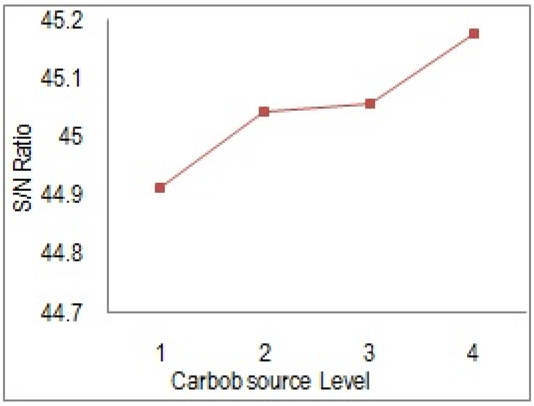

Fig. 3- Effect of Carbon source on S/N ratio

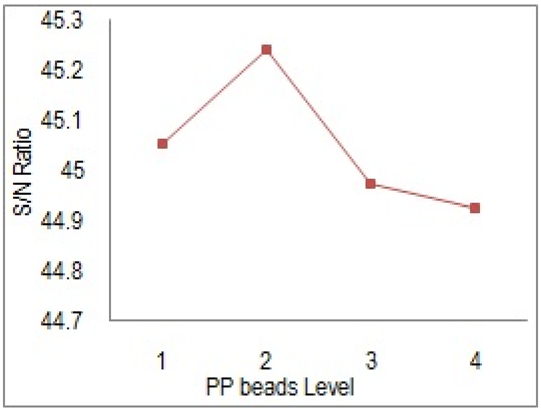

Fig. 4- Effect of PP beads on S/N ratio

Confirmation Experiment

Confirmation test is a crucial step recommended by Taguchi to verify experimental conclusions. The purpose of the confirmation experiment is to validate the conclusions drawn during the analysis phase. Two tests were conducted to verify the initial nitrate removal at optimum level of A_2 B_3 C_4 D_2 E_2 and values obtained were 188.016 and 191.024 respectively, with an average of 189.52 and compared with the result obtained from the predictive equation as shown in Table 5. The experimental values agree reasonably well with predictions because an error of 2.266 for the S/N ratio is observed when predicted results are compared with experimental values. However, the errors can be further reduced if the number of measurements is increased.

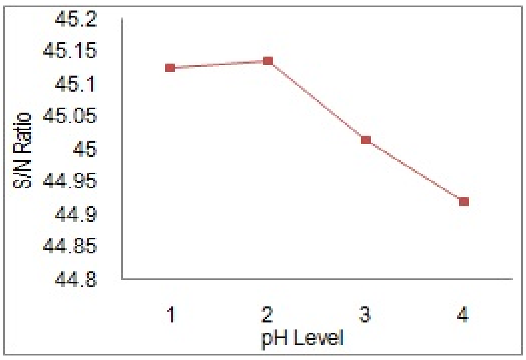

Fig. 5-Effect of pH on S/N ratio

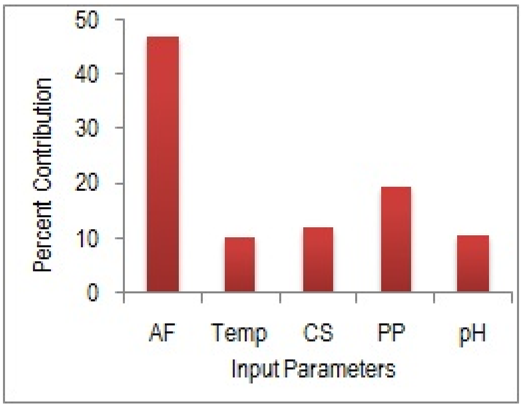

Fig. 6-Percentage contribution of parameters

Table-5-Results of the confirmation experiment:

	Optimal parameter		
	Prediction	Experimental	Diff.
Level	$A_2 B_3 C_4 D_2 E_2$	$A_2 B_3 C_4 D_2 E_2$	
Nitrate removal (ppm)	194.066	189.52	4.546
S/N ratio	45.759	43.493	2.266

CONCLUSION

Taguchi method is an effective tool for optimizing the parameters for the denitrification of wastewater and the procedure reduces the practical performances for experimentation and the approach is easy to implement. The present paper presents the optimal condition for denitrification of waste water using *Pseudomonas stutzeri* microorganism in a fluidized bed bio reactor at 200 ppm. The optimum conditions are A_2 B_3 C_4 D_2 and E2, i.e. Airflow rate = 2.5 lpm, Temperature = 30^0 C, Carbon source = 85 mg/lit, Poly propylene (PP) beads=15 gm/lit and pH=7. It is observed that the nitrate removal efficiency is more than 96% at this optimum condition. The ANOVA method is also used for the individual parameter percentage contribution and found that Airflow rates contribution is more compared to other parameters and temperature provides the least contribution.

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