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Optimization of culture condition for growth and phenol degradation by *Alcaligenes faecalis* JF339228 using Taguchi Methodology

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ABSTRACT

The optimization of five process parameters such as pH, agitation, temperature, inoculum percentage and incubation time were optimized by Taguchi robust design method for obtaining enhanced biomass and phenol degradation by the isolated *Alcaligenes faecalis* JF339228 from Durgapur steel industry (DSP), India. About 18 experiments were conducted with a different combination of factors and the results obtained in terms of growth of specific bacterial strain and phenol degradation rates were processed in the Qualitek-4 software to study the main effect of individual factors. The main effect, interaction effects and optimal levels of the process factors were determined using signal-to-noise (S/N) ratio. The effect of factors has been studied for bacterial growth and phenol degradation by *A. faecalis* JF339228. Optimization of the said parameters has been evaluated by Taguchi method and analysed by analysis of variance. Predicted results showed enhanced process performance such as biomass (131.78%) and phenol degradation (130.40%) with pH (7), temperature (37.5˚C), agitation (150 rpm), inoculum percentage (6%) and incubation time (72 h). The optimum values of medium composition obtained for biochemical degradation of phenol by *A. faecalis* JF339228 could be used for phenol degradation using optimized process parameter by Taguchi's by this microorganism.

Keywords: Optimization; Phenol degradation; Taguchi robust design; Biomass and *Alcaligenes faecalis* JF339228

1. Introduction

Phenol and its derivatives are the most common representatives of the toxic organic pollutants, being produced in several industries and operations such as gas and coke oven industries, polymeric resin production, petroleum refineries, fiber glass units, pharmaceuticals, explosive manufacturing, plastic and varnish industries, and textile industries, making use of

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organic dyes, smelting and related metallurgical operations, etc. [1–4]. In addition to being potential carcinogens, phenols are toxic or lethal to fish at relatively low levels of 5–25 mg/L [2,5–7]. Once the phenol bearing wastewater is chlorinated, toxic polychlorinated phenols formed create an objectionable test and odors to drinking water even at a very low level of 2.0 μg/L [8]. Therefore, to save our environment, ecosystem aquatic life and fauna flora, it has been mandatory for all industries to treat the phenol and its derivatives containing wastewater before disposal. The pretreatment alternatives such as adsorption [9–11], ion exchange [12], membrane separation [13–15], advanced oxidation process [16–18] and solvent extraction [19] often suffer from serious drawbacks including high cost and some cases the formation of hazardous by products [20]. The bioactive activated carbon (BAC) treatment in removing total phenols has been found to be satisfactory [21]. The potential of the diverse group of microorganisms, including bacteria [22–24], yeasts [25,26], algae [27], filamentous fungi [28] and enzymatic process [29] are being studied for their metabolic capacities to degrade phenol. Though the microbial treatment as one of the biodegradation step is environmental friendly and cost effective [30–33], but it has some limitations too as it requires the presence of specific microbial isolate/enzymes tolerant to higher concentration of pollutants and the knowledge of factors that influence the growth and metabolism of phenol. Microbial degradation process results from the combined enzyme, cell and community based activities of microorganisms whose regulation and kinetics are likely to be highly individualized and variable in space and time. The activities are dependent on environmental factors, including nutrient levels, the availability of alternative substrate, predation, pollution density, contaminant substrate concentration, pre-exposure, and bioavailability. The process parameters like temperature, pH, viscosity, dissolved oxygen concentration, etc., also play a key role in this context [34–39]. All these factors in combination make it difficult to predict the fate of contaminant or to interpret biodegradability data from natural samples. It has been observed that enzyme activities may reflect microbial activities in wastewater [40]. Kibret et al. [6] characterized the activity of activated sludge from a laboratory scale treatment plant, which was subjected to stepwise increases in the inlet phenol concentration, by the assay of catechol 1,2-dioxygenase activity, which was dependent on the concentration of phenol in the system. Phenol is metabolized by two major metabolic pathways known as ortho and meta cleavage [41]. Phenol hydroxylase represents the first enzyme in the metabolic pathway of phenol degradation [42]. In the next step, two enzymes can be induced, catechol 1,2- or 2,3 dioxygenase, present in the ortho and meta-pathways, respectively [41,42]. The natural biodegradation process enzymatically uses organic waste as a source of carbon and energy and chemically converts these wastes into new cell mass and nontoxic by-products. Microbial cells generally obtain these vital nutrients directly from water, soil, sludge and sediment. However in many cases, the material undergoing bioremediation is deficient in one or more necessary compounds, and unless the correct nutritional balance is maintained in the bio-treatment with optimized operational condition, microbial activity may slow down due to the lack of vital nourishment and proper friendly environment. Secondary pollution may generate due to the lack of proper nutrients and operational conditions. Optimization of these factors are thus very much necessary to enhance the process for degradation of phenol in wastewater [43,44].

In conventional method, numerous experiments have to be carried out to optimize all the parameters (factors) and to establish the best possible conditions by interrelating all the parameters. This method of studying one variable at a time is time consuming, cumbersome, requires more experimental data sets and cannot provide information about the mutual interactions of the parameters [45]. On the other hand, traditional DOE (experimental design) focuses on the average process performance characteristics. It concentrates on the effect of variation on the process characteristics [46,47].

Presently literature review reveals that Taguchi method has been widely used to optimize the reaction variables in biochemical processes. This approach involves the study of a given system by a set of independent variables (factors) both controllable and uncontrollable (dynamic/noise) over a specific region of interest (levels) [48]. It is also noted that the small scale experiments are valid over the entire experimental region [46]. Experimental data was analyzed by using the ANOVA (analysis of variance) and factors (physical parameters) give the output whose effects are statistically significant in finding the optimum levels.

The objective of the present study is to optimize the process parameters (temperature, pH, etc.) for enhanced phenol degradation by *Alcaligenes faecalis* JF339228 using Taguchi orthogonal array (OA), a factorial based approach.

2. Materials and methods

2.1. Chemicals and analysis

Methanol for HPLC (E-Merck, Germany) and Phenol for HPLC (E-Merck, Germany), were used in

this study. Water used for the HPLC analysis was prepared by Ultrapure Water System (Arium®, 611UF, Sartorius, Germany). All other chemicals used were of analytical grade commercially available in India.

2.2. Microorganism and seed culture

Phenol degrading *A. faecalis* JF339228 was isolated from Durgapur (India) steel plant wastewater. The microorganism was grown on nutrient agar slants at 37°C at pH 7.4. The composition of nutrient agar is beef extract (1.5 g/L), yeast extract (1.5 g/L), sodium chloride (5 g/L), peptic digest of animal tissue (5 g/L) and agar (15 g/L). *A. faecalis* JF339228 was maintained by sub-culturing on nutrient agar slants kept at pH 7.4. For further experiments, the culture was revived by adding a loop full of pure culture into 50 mL of sterile nutrient broth (pH 7.4).

2.3. Taguchi Methodology

Biochemical degradation of phenol is very much dependent on process parameters and incubation time. Taguchi method involves the establishment of different experimental situations through orthogonal arrays (OAs) to reduce experimental errors and to enhance the efficiency and reproducibility of experiments. Robust design has been considered in this study as it helps to minimize the effect of noise factor in the process of optimization and leads to a dynamic or robust experimental design [49]. There are four phase for Taguchi Methodology study. They are planning, conducting, analysis and validation. The schematic representation of the planned methodology has been shown in Fig. 1. Each phase is separated with distinct objective and is connected sequence wise to achieve the overall optimization process.

2.3.1. Design of experiments (Phase 1)

The first step in phase-1, various process parameters were selected, having a critical effect on microbial growth and phenol degradation. All the variables were investigated within the feasible range so that the variation inherent in the process does not mask the factor effect. In this study, pH, temperature, agitation, inoculum percentage and incubation time were considered as important factors for growth and phenol degradation by *A. faecalis* JF339228 [50]. All the variables were investigated at three widely spaced levels shown in Table 1 based on our previous work. In the next step, matrix was designed with the appropriate

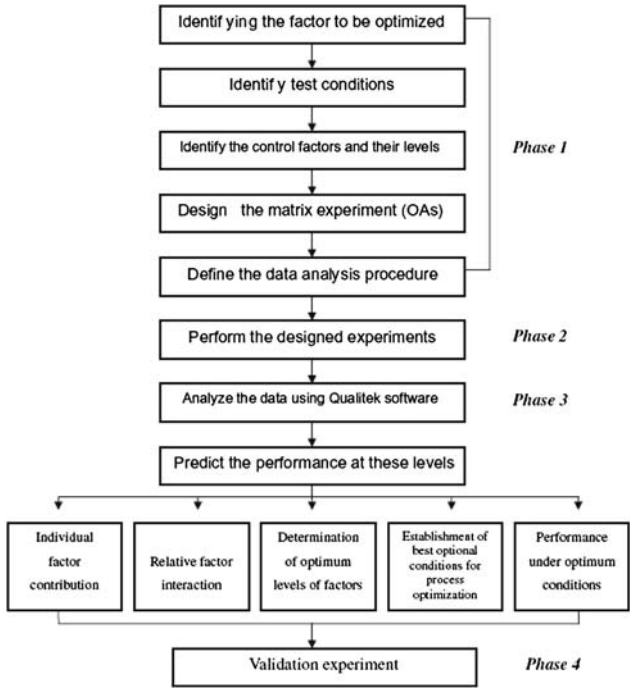

Fig. 1. Schematic representation of the steps involved in the Taguchi DOE methodology.

OAs for the selected parameters and their levels. Taguchi provides many standard OAs and corresponding linear graphs for this purpose [51]. In the present study, three levels of ten factors (Tables 1 and 2) biodegradation were considered and the size of experimentation was represented by symbolic array of L-18 (which indicated 18 experimental trials).

2.3.2. Growth and phenol degradation with selected factors and levels (Phase 2)

A fresh seed culture was inoculated in 250 mL flask with 50 mL simulated media, containing (g/L) of phenol, 2.1; phosphate, 0.42; iron sulphate, 0.46; calcium chloride, 0.08; magnesium chloride, 0.18 and ammonium sulphate, 0.25. Biodegradation was performed at different pH (6, 7 and 8), temperatures (35, 37.5 and 40°C), agitation (120–180 rpm), inoculum percentage (2%, 4% and 6% v/v) and incubation time (48, 60 and 72 h). The culture was centrifuged at 8000 rpm for 10 min at 4°C. The cell pellet was used for growth study and the supernatant was used for residual phenol analysis.

The supernatant was filtered in 0.45 μm nylon membrane filters, diluted with water, and analyzed. For the analysis of sample, Waters™ 600 Pump based HPLC system equipped with Waters quaternary pump, Waters manual injector, Waters on-line degasser AF,

Table 1
Experimental range of the five numerical variables studied using Taguchi Methodology

Serial no.	Factor code	Factor	Level-1	Level-2	Level-3
1	*A*	pH	6	7	8
2	*B*	Temperature (°C)	35	37.5	40
3	*C*	Agitation (rpm)	120	150	180
4	*D*	Inoculum percentage (%)	2	4	6
5	*E*	Incubation time (h)	48	60	72

CTO-10 AS VP column oven and Waters 2489 UV/Visible Detector was used. Water Empowered software (Version: Empower 2 software Build 2154) was used for data acquisition and mathematical calculations. Chromatographic separation of phenol was performed on a C_{18} hypersil column (4.6 mm×250 mm; 5 μm particle size; Waters, USA). Mobile phase used was methanol–water (5% v/v acetic acid) (60:40 v/v), at a flow rate of 1 mL/min. Temperature of the column oven was maintained at 30°C. The sample (20 μL) was injected and analyzed at 270 nm using UV–Visible detector. The sample was collected from different media condition as describe Table 2, where initial pH was different. During HPLC analysis the single peak of phenol was found though the pK_a value of phenol was 9.95. The standard curve of phenol was made at different pH. It is obvious that some fraction of phenol molecule is in basic form at different pH but no separate peak was found during HPLC analysis. Though they are in different form yet they are eluted in same time in our HPLC system. So the effect of phenol molecules that were in basic form, have no effect on the overall degradation calculation. This indicates that HPLC mobile phase has no influence on the acido-basic forms.

For dry cell weight (DCW) estimation, the cell Pellet was washed twice with distilled water and then dried in a hot air oven at 105°C. All experiments were done in triplicate.

2.3.3. Data analysis and prediction of performance (Phase 3)

The experimental data obtained was processed using Qualitek-4 software (Nutek Inc., MI, USA) to evaluate the influence of individual factors, multiple interaction of the selected factors, determination of optimum conditions and the process performance on microbial growth and phenol degradation. In the present study S/N analysis was employed with bigger-is-better performance characteristics for all the experimental cases. In the Taguchi method, the term 'signal' represents the desirable value (mean) and the term 'noise' represents the undesirable value (SD) for the output characteristic [52]. Therefore, the signal-to-noise (S/N) ratio is the ratio of the mean to the SD. Taguchi used the S/N ratio to measure the quality characteristic deviating from the desired value. A loss function [$L(y)$] is developed for the deviation [48] as represented by $L(y) = k \times (y - m)^2$, where k denotes the proportionality constant, m represents the target value and y is the experimental value obtained for each trail. In case of bigger and better quality characteristics the loss function can be written as $L(y) = k \times (1/y^2)$ and the expected loss function can be represented by

$$E[L(y)] = kE(1/y^2) \tag{1}$$

where $E(1/y^2)$ can be estimated from a sample of n as

$$\sum_{i=1}^{n} [1/y_i^2]/n \tag{2}$$

Taguchi used the signal-to-noise (S/N) ratio as a performance measurement of a dynamic system to evaluate the robustness of the overall process [53]. The mathematical expression for the S/N ratio for the "bigger is better" case for the performance statistics that measure deviation from the target, called as mean square deviation (MSD) was given by

$$Z = -10\log(\text{MSD}) = -10\log\sum_{i=1}^{n} [1/y_i^2]/n \tag{3}$$

2.3.4. Validation of the experimental model

In order to validate the methodology, experiment was further performed for growth and phenol degradation using the predicted optimized culture conditions.

Table 2
L_{18} OA (3^5) of design experiments for five variables with actual growth and phenol degradation

Number of experiment	A	B	C	D	E	Biomass (g/l) (triplicate values)			Phenol degradation (%) (triplicate values)		
1	1	1	1	1	1	0.43	0.41	0.45	19.28	18.32	20.25
2	1	2	2	2	2	1.12	1.06	1.18	50.13	47.62	52.64
3	1	3	3	3	3	1.03	0.98	1.08	48.59	43.96	46.27
4	2	1	1	2	2	0.94	0.90	0.99	41.26	37.33	39.29
5	2	2	2	3	3	2.19	2.08	2.30	100.97	91.35	96.16
6	2	3	3	1	1	0.82	0.77	0.86	35.18	31.83	33.51
7	3	1	2	1	3	1.04	0.99	1.09	48.84	44.18	46.51
8	3	2	3	2	1	0.94	0.89	0.98	44.03	39.83	41.93
9	3	3	1	3	2	1.02	0.97	1.07	47.81	43.27	45.55
10	1	1	3	3	2	0.71	0.67	0.75	33.41	30.23	31.81
11	1	2	1	1	3	1.08	1.02	1.13	45.78	48.19	50.61
12	1	3	2	2	1	0.65	0.61	0.68	28.92	27.47	30.37
13	2	1	2	3	1	1.09	1.04	1.15	47.00	44.66	49.35
14	2	2	3	1	2	1.31	1.25	1.38	54.71	51.97	57.45
15	2	3	1	2	3	1.31	1.25	1.38	53.75	51.06	56.45
16	3	1	3	2	3	1.12	1.07	1.18	50.25	47.73	52.75
17	3	2	1	3	1	1.01	0.96	1.07	45.43	43.15	47.70
18	3	3	2	1	2	0.99	0.94	1.04	44.34	42.11	46.56

3. Results and discussion

At present, the biological phenol degradation is very important in chemical process industries. So, in this study, a systematic and robust optimization strategy was adopted to find out the optimum critical process parameters for enhanced phenol degradation.

3.1. Taguchi Methodology

3.1.1. Influence of individual factors

The influence of each factor at the assigned levels on growth and phenol degradation by *A. faecalis* JF339228 has been represented in Table 2. Results obtained with the designed experimental sets (Table 3) showed that the process efficiency was very much dependent on the selected culture conditions. Individually, agitation, temperature and incubation time were very influential at level 1, level 2 and level 3, respectively on microbial growth and phenol degradation. The magnitude of difference between the average effects (L_2–L_1) represents the relative influence of the factor or interaction to the variability of results. The larger the difference, the stronger is the influence [51]. Further it has been observed from Table 3 that among the studied factors, pH showed stronger influence on the growth of bacteria followed by temperature, incubation time, agitation, and inoculum percentage. But for phenol degradation, temperature showed stronger influence followed by pH, incubation time, agitation, and inoculum percentage. The present findings showed that bacterial growth and phenol degradation were greatly influenced by process parameters.

Microbial growth and phenol degradation were controlled by both pH and temperature. Maximum growth and phenol degradation were found at pH 7.0 and temperature at 37.5˚C, at level 2. The yield was found to be decreased in any other level of these factors. This indicates that the pH below or above 7.0 may affect the microbial growth due to the variation of H^+ and OH^- ion in the system. In addition, the variation of hydrogen ion in the system may affect the enzyme activity for phenol degradation as well as active transportation of food and other micronutrients in between cell and environment and eventually effecting the phenol degradation.

While optimizing the temperature, below and above the optimum temperature, the activity of microbial cell seems to be reduced effecting the microbial phenol degradation efficiency. In the present study, 37.5˚C is the optimum temperature. Another important parameter is the O_2 transport in reaction system [6]. For improving the microbial growth, O_2 transport plays an important role, so the shaking speed (rpm)

Table 3
Main effects of selected factors

Serial no.	Factor code	Factor	Effect of individual factors in biomass production analysis by S/N ratio				Effect of individual factors in phenol degradation analysis by S/N ratio			
			Level 1	Level 2	Level 3	L2 – L1	Level 1	Level 2	Level 3	L2 – L1
1	*A*	pH	−2.06	1.65	0.14	3.71	30.97	34.12	33.16	3.15
2	*B*	Temperature (°C)	−1.47	1.7	−0.5	3.17	31.39	34.59	32.27	3.2
3	*C*	Agitation (rpm)	−0.78	0.82	−0.3	1.6	32.01	33.75	32.49	1.74
4	*D*	Inoculum percentage (%)	−0.99	−0.11	1.87	0.88	31.8	32.68	33.77	0.88
5	*E*	Incubation time (h)	−2.12	−0.03	1.88	2.09	30.72	32.78	34.75	2.06

of flask was another parameter which effects the uniform mixing of reactants, well distribution of O_2. With increasing rpm uniformity is achieved in the reaction vessel and cause of well distribution of O_2, substrate, microbes and product which leads to enhance the efficiency of the process as a aerobic phenol degradation, but excess rpm of liquid cause of extra shear force (friction between the liquid layers of reactant medium) may lead to damage the microbial cell present into the media. The optimum value was obtained 150 rpm for *A. faecalis* JF339228 in the present study for phenol degradation.

Extra inoculums value help to reduce the lag phase of any microbial growth [54]. In the present case, thus with increasing inoculums volume of *A. faecalis* JF339228 a better phenol degradation is achieved initially, but above 6% (v/v) the effect in phenol degradation is not significant. Thus the optimum inoculums value of *A. faecalis* JF339228 is 6% for minimum phenol degradation in a shake flask method. It may be due to the effect of pH and temperature on growth and phenol degradation. The highest microbial growth and phenol degradation was found with the increase of agitation up to level 2 but in level 3 it (slow down) shows negative effect. This was demonstrated by the fact that, agitation raises the oxygen mass transfer [55]. Biomass and phenol degradation was also influenced by the initial inoculum concentration. Maximum yield was noticed at the level 3 of inoculum. Result showed that higher bacterial growth and phenol degradation were found with subsequent increase of incubation time up to level 3.

3.1.2. Influence of factors interaction

The severity index (SI) was evaluated from Taguchi DOE that represents the influence of two individual factors at various levels of interaction (Table 4). In this table, the 'columns' represents the locations to which the interacting factors are assigned. The 100% SI indicates 90° angle between the lines (factors), while 0% SI for parallel lines. Reserved column' shows the column that should be reserved if this interaction effect has to be studied. 'Levels' indicate the level of factors desirable for the optimum conditions.

The highest interaction (SI 40.69%) was observed in between agitation and inoculum percentage followed by inoculum percentage and temperature (SI 36.26%), incubation time and inoculum percentage (SI 20.23%), pH and incubation time (SI 15.43%), agitation and incubation time (SI 10.35%), pH and inoculum percentage (SI 9.17%), pH and temperature (SI 7.75%), pH and agitation (SI 6.45), temperature and agitation (SI 6.11) and incubation time and temperature (SI 2.63%) on biomass production (Table 4). From the Table 3, it is assumed that pH is highest impact factor followed by temperature (high impact factor), incubation time (moderate impact factor), agitation (low impact factor) and inoculum percentage (lowest impact factor) for biomass production. It was interesting to note that agitation and inoculum percentage were low and lowest impact factor, respectively but had shown highest severity index in combination. Temperature is high impact factor, shows comparatively high severity index in combination with inoculum percentage (lowest impact factor). Incubation time is moderate impact factor but shows higher severity index in combination with inoculum percentage (lowest impact factor). pH is highest impact factor but showed comparatively higher severity index in combination with inoculum percentage (lowest impact factor). Agitation and inoculum percentage are low and lowest impact factor but a good severity index was found in combination of them.

On the contrary, a lowest severity index (2.63%) was found with temperature (high impact factor) and incubation time (moderate impact factor). SI (6.11%) between temperature (high impact factor) and

Table 4
Estimated interaction of severity index for two factors

Serial no.	Biomass production					Phenol degradation				
	Factors	Columns	SII (100%)	RC	Levels	Factors	Columns	SII (100%)	RC	Levels
1	Agitation × inoculum percentage	4 × 5	40.69	1	[2,3]	Agitation × inoculum percentage	4 × 5	37.08	1	[2.3]
2	Temperature × inoculum percentage	3 × 5	36.26	6	[2,3]	Temperature × inoculum percentage	3 × 5	32.07	6	[2,3]
3	Inoculum percentage × incubation time	5 × 6	20.23	3	[3,3]	Inoculum percentage × incubation time	5 × 6	21.70	3	[3,3]
4	pH × incubation time	2 × 6	15.43	4	[2,3]	pH × incubation time	2 × 6	16.61	4	[2,3]
5	Agitation × incubation time	4 × 6	10.35	2	[2,3]	pH × agitation	2 × 4	9.99	6	[2,2]
6	pH × inoculum percentage	2 × 5	9.17	7	[2,3]	pH × inoculum percentage	2 × 5	9.57	7	[2,3]
7	pH × temperature	2 × 3	7.75	1	[2,2]	pH × temperature	2 × 3	7.53	1	[2,2]
8	pH × agitation	2 × 4	6.45	6	[2,2]	Temperature × agitation	3 × 4	7.3	7	[2,2]
9	Temperature × agitation	3 × 4	6.11	7	[2,2]	Agitation × incubation time	4 × 6	6.83	2	[2,3]
10	Temperature × incubation time	3 × 6	2.63	5	[2,3]	Temperature × incubation time	3 × 6	1.31	5	[2,3]

Table 5
Analysis of variance

Factor	Biomass Production						Phenol degradation					
	DOF	SS	Variance	F-ratio	Pure sum	Percent (%)	DOF	SS	Variance	F-ratio	Pure sum	Percent (%)
pH	2	41.80	20.90	80.68	41.29	29.21	2	31.30	15.65	59.62	30.78	22.63
Temperature (°C)	2	31.67	15.83	61.12	31.15	22.04	2	32.86	16.43	62.70	32.34	23.78
Agitation (rpm)	2	8.04	4.02	15.51	7.52	5.32	2	9.67	4.84	18.43	9.15	6.73
Inoculum percentage (%)	2	10.04	5.02	19.37	9.52	6.73	2	11.78	5.89	22.43	11.25	8.27
Incubation time (h)	2	48	24.00	92.63	47.48	33.59	2	48.56	24.28	92.50	48.03	35.32
Other/error	7	1.81	0.26			3.12	7	1.84	0.26			3.28
Total	17	141.36				100	17	130.01				100

agitation (low impact factor) was significantly low. pH (highest impact factor) with agitation (low impact factor) showed significant low SI (6.45%).

In phenol degradation, the highest interaction (SI 37.08%) was observed in between agitation and inoculum percentage followed by inoculum percentage and temperature (SI 32.07%), incubation time and inoculum percentage (SI 21.07%), pH and incubation time (SI 16.61%), pH and agitation (SI 9.99%), pH and inoculum percentage (SI 9.57%), pH and temperature (SI 7.53%), temperature and agitation (SI 7.3%), agitation and incubation time (SI 6.83%) and incubation time and temperature (SI 1.31%) (Table 4). From the Table 3, it is assumed that temperature is highest impact factor followed by pH (high impact factor), incubation time (moderate impact factor), agitation (low impact factor) and inoculum percentage (v/v) (lowest impact factor) for phenol degradation. Agitation and inoculum percentage are low and lowest impact factor, respectively but higher severity index is found in combination. Inoculum percentage (lowest impact factor) in combination with temperature (high impact factor), incubation time (moderate impact factor) showed higher severity index.

pH is the highest impact factor but has shown higher severity index in combination with incubation time, agitation, inoculum percentage and temperature where incubation time, agitation, inoculum percentage and temperature are moderate, low, lowest and high impact factors, respectively. On the contrary, temperature (high impact factor) showed comparatively lower severity index in combination with agitation (low impact factor). Agitation is moderate impact factor showed good severity index in combination with incubation time (moderate impact factor). Temperature is the high impact factor but showed lowest SI (1.31%) with incubation time (moderate impact factor). It is evident from the results that the% phenol degradation is quite dependent of the individual influence and also on interaction of factors.

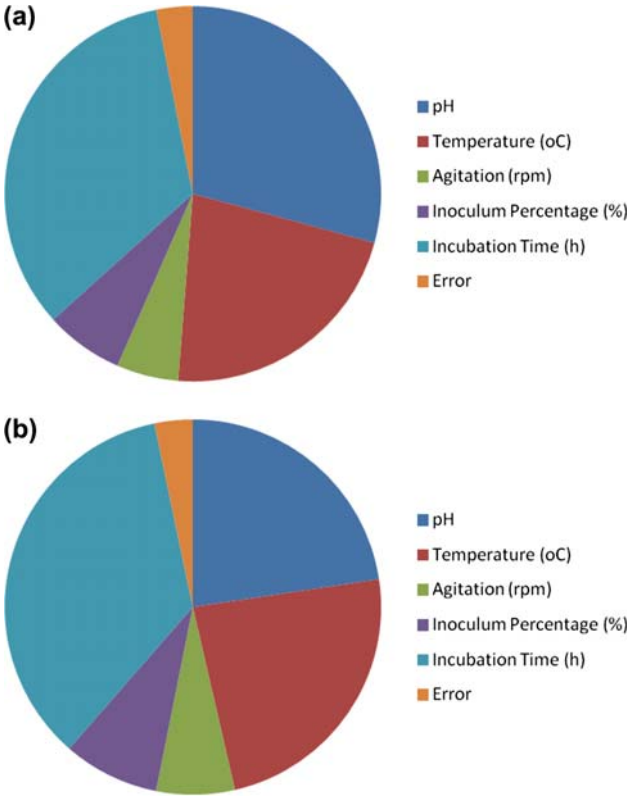

Fig. 2. Relative influence of factors and interaction for (a) biomass and (b) phenol degradation.

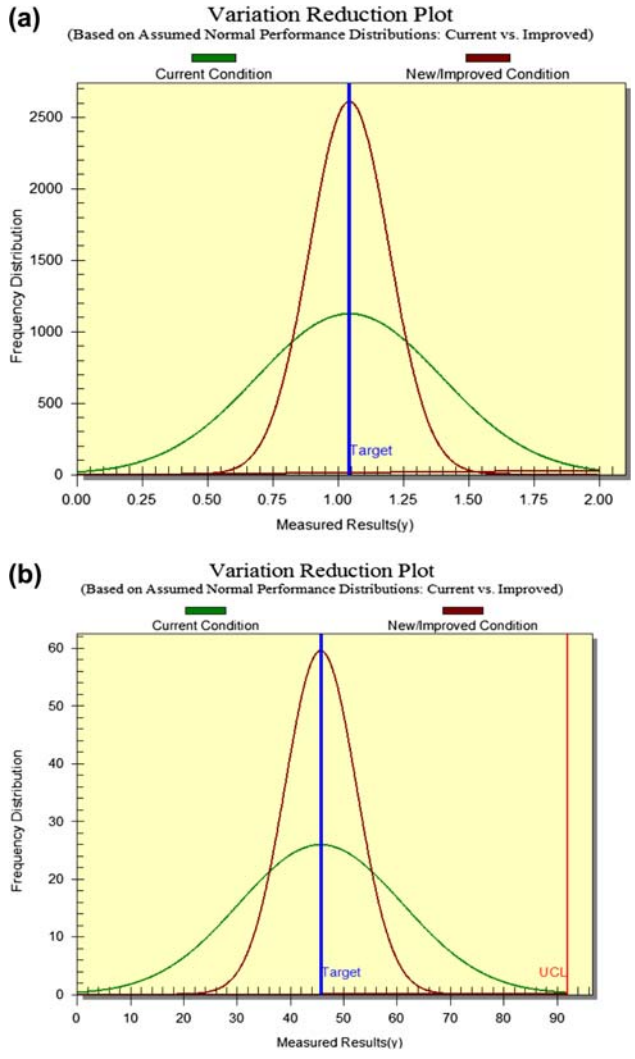

Fig. 3. Performance distribution of current and improved condition for (a) biomass and (b) phenol degradation.

Table 6
Optimum condition for biomass and phenol degradation

Factor code	Factor	Values	Level	Contribution for biomass production from S/N ratio	Contribution for phenol degradation from S/N ratio
A	pH	7	2	1.74	1.37
B	Temperature (°C)	37.5	2	1.79	1.84
C	Agitation (rpm)	150	2	0.90	1.00
D	Inoculum percentage (%)	6	3	0.92	1.02
E	Incubation time (h)	72	3	1.97	2.00
	Total contribution from all factors			7.32	7.23
	Current grand average performance			−0.09	32.75
	Expected result at optimum condition			7.24	39.98

3.2. ANOVA

Analysis of variance (ANOVA) was used to analyze the experimental data and to determine the variation of result due to each factor. Based on F ratio, it was observed that all factors and interactions were statistically significant within 95% confidence level. ANOVA with the percentage of contribution of each factor with interactions are shown in Table 5. Results showed that incubation time contributed the maximum impact (33.59%) on biomass production followed by pH (29.21%), temperature (22.04%), inoculum percentage (6.73%) and agitation (5.32%). Incubation time (35.32%) was also the most significant contributory factor for phenol degradation. The next significant factors for phenol degradation in order of importance were temperature (23.78%), pH (22.63%), inoculum percentage (8.27%) and agitation (6.73%).

Fig. 2(a) and (b) showed the variation of biomass and phenol degradation at chosen levels of each significant factor. Individually, each significant factor influenced production of biomass and phenol degradation at certain level. However, in combination, the level of significant factors was different for maximum yield, which might be reasoned due to the interactive effect of different factors.

3.3. Optimum process parameters

Optimum condition of significant factors and their performance in terms of contribution for achieving higher biomass and phenol degradation were shown in Table 6. It can be seen from the Table 5 that incubation time showed the maximum role on biomass production and phenol degradation followed by temperature, pH, inoculum percentage and agitation. Taguchi DOE design suggested that the higher levels of biomass and phenol degradation can be achieved with pH (7), temperature (37.5°C), agitation (150 rpm) and incubation time (72 h) in the present case. The expected biomass and phenol degradation at optimum condition in S/N ratio were found to be 7.24 and 39.98, respectively (total contribution from all the factors being found 7.32 and 7.23 with grand average performance of −0.088 and 32.75, respectively). The estimated biomass and phenol degradation from the S/N ratio were 2.3 g/L and 99.76% with MSD of 0.18893 and 0.0001, respectively (by Eq. (3)).

3.4. Validation experiments

The distribution of current condition along with improved condition is shown in Fig. 3(a) and (b). It was evident from the figure that the biomass yield could be increased from 0.99 to 2.31 g/L. The overall 132.40% enhancement in the biomass yield could be achieved. Phenol degradation could be augmented from 43.40% to 99.76%. The overall 129.85% enhancement in the phenol degradation could be attained. Further to validate the proposed experimental methodology, phenol degradation experiments were performed by employing the obtained optimized culture conditions. The experimental data showed that enhanced biomass (131.78%) and phenol degradation (130.40%) were found with the optimized culture conditions.

4. Conclusion

Taguchi Methodology has been successfully applied to optimize the process parameters for enhanced biomass and phenol degradation using *A. faecalis* JF339228 and was experimentally verified. The Taguchi Methodology predicts a maximum biomass (131.78%) and phenol degradation (130.40%) can be achieved in the optimized conditions. The

main effect, interaction effects and the optimal levels of the culture condition were determined using S/N ratio.

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