ATIPC – 2020 Special Issue

J. Indian Chem. Soc.,
Vol. 97, No. 12b, December 2020, pp. 2833-2839

Parametric optimization by Taguchi orthogonal array methodology for enhanced biodegradation of 4-chlorophenol by an isolated bacterial consortium

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Manuscript received online 10 December 2020, accepted 31 December 2020

Statistical optimization of different parameters for enhanced degradation of 4-chlorophenol (4-CP) by an isolated consortium was conducted using Taguchi Orthogonal Array (TOA) methodology. Five parameters and their three levels were selected based on their crucial impact on 4-CP biodegradation and microbial growth. Qualitek-4 software was used to perform the optimization experimentation. L-18 Orthogonal Array was designed by the Taguchi methodology under which the test runs for the study were conducted. The Signal to Noise (S/N) ratio having the greater, the better characteristics was used as a measure to assess the impact of individual factors and their interaction on the process under investigation. The optimum conditions established by TOA are 32°C temperature, 7.5 pH, 140 rpm agitation, 0.6 OD_{600} inoculum dose and, 72 h incubation time. Validation experiments revealed the attainment of 99.16% 4-CP biodegradation and 0.587 g/L biomass concentration under the optimized conditions, thereby enhancing 4-CP biodegradation and biomass concentration from the current conditions by 138.42% and 107.42%, respectively. This study confirms the feasibility of the application of the consortium in the treatment of 4-CP laden wastewater streams.

Keywords: Biodegradation, 4-chlorophenol, optimization, orthogonal array, Taguchi methodology.

Introduction

Industrialization and urbanization have led to the rise in the manufacture of various anthropogenic compounds that have disrupted ecological harmony. Chlorophenols are one such class of hazardous compounds that have widespread applications in paper and pulp industries, leather tanneries, dyes and herbicides, and chlorination for disinfection of potable and wastewaters[1]. The primary source of water contamination by the chlorophenolic compounds is the discharge of industrial effluents and leaching from landfills[2]. These pose serious health hazards due to their toxic nature and recalcitrance in the environment[3]. Due to their recalcitrance, chlorophenols are prone to accumulate in nature and cause soil and water contamination[4]. Chlorophenols are mutagenic[5,6] and cause profound health implications on flora and fauna. Due to their lipophilic nature, they can gain entry through the cell membrane and bio-magnify among aquatic biota[7]. The toxicity of 4-CP necessitates its mitigation from the industrial effluents before their disposal into the environment[8].

Various physicochemical techniques, including adsorption, oxidation, solvent extraction, volatilization, photo-decomposition, and electrochemical methods, have been used for the decontamination of such xenobiotics[9]. However, these conventional detoxification methods suffer from certain limitations, including high price, low elimination competence, and toxic by-products accumulation[8]. Therefore, detoxification of chlorophenols via biological routes has been envisaged as a promising alternative due to their eco-friendly and economical approach[10]. There are several reports on 4-chlorophenol biodegradation by pure cultures[11–13], mostly at lower concentration owing to its inhibitory nature at higher concentration. Reports on the biodegradation of 4-chlorophenol by a microbial consortium are relatively limited in the literature. Microbial consortia are generally preferred because of the faster rate of degradation and potential to eliminate a diverse range of pollutants owing to the presence of synergistic relationship or mutual cooperation between multiple microbial partners that facilitate removal of toxic compounds whose removal by pure cultures would otherwise have been difficult[14,15]. The cause of the enhanced degradation potential of the microbial consortium is the combined action of the catabolic enzymes present in the consortial partners[16]. Several studies have elucidated the potential of microbial consortia in eliminating toxic anthropogenic compounds[15,17–19].

Enhanced degradation of pollutants requires the establishment of optimum physicochemical parameters. The conventional optimization procedures such as the change of one variable per time approach (COVT) is a time-consuming and cumbersome method that requires numerous experiments to be performed when the number of factors is high and does not accord focus on the mutual interactions between the parameters. Also, the establishment of a global optimum is not achieved[20–22]. These bottlenecks of the traditional optimization procedures can be mitigated by using statistical approach-based optimization tools involving Design Of Experiments (DOE) such as the Taguchi Orthogonal Array method (TOA). The Taguchi optimization technique is a robust method of reducing process variations. It involves investigating the process performance by analyzing the most crucial factors affecting the process and their mutual interactions[23,24]. It is a powerful optimization tool that facilitates optimization with fewer experimental trial runs[25]. The advantages of Taguchi DOE include lesser cost, quality improvement, and robust design output. This technique aids in the identification of the effects of individual factors, their mutual interactions, and the association between the variables and the operational conditions[25].

Therefore, in our study, we have attempted to optimize the physicochemical parameters and investigate the measure of the influence of the individual parameters using the Taguchi OA technique to maximize 4-CP degradation by a previously isolated microbial consortium[26].

Materials and methods

Chemicals:

4-Chlorophenol (4-CP) of analytical grade was procured from Himedia (India). The other chemicals used in this study were of the highest purity available and purchased from Sigma-Aldrich and Himedia.

Microorganisms and media composition:

A metabolically versatile bacterial consortium was isolated in our previous study[26]. The isolated consortium comprised of four bacterial strains, namely *Brevibacterium* sp. PNP1 (MH169212), *Pseudomonas* sp. PNP2 (MH169213), *Agromyces mediolanus* PNP3 (MH169214), and *Microbacterium oxydans* PNP4 (MH169215). Minimal Salt Medium (MSM) with the following composition (g/L) was used in this study 1.2KH_2PO_4, 4.8K_2HPO_4, 0.25$MgSO_4.7H_2O$, 0.0025$FeSO_4.7H_2O$, 0.026$CaCl_2.2H_2O$[27] and maintained at pH 8. The consortium was grown in MSM amended with 500 mg/L of 4-CP as the sole carbon source at 32°C for 72 h and used as the pre-culture (inoculum) for the subsequent biodegradation experiments for the optimization studies.

Table 1. List of five parameters and their levels

Sl. no.	Factor code	Factor	Level-1	Level-2	Level-3
1.	A	Temperature (°C)	30	32	34
2.	B	pH	6.5	7.5	8.5
3.	C	Agitation (rpm)	120	140	160
4.	D	Inoculum dose (OD_{600})	0.2	0.4	0.6
5.	E	Incubation time (h)	24	48	72

Statistical optimization of the parameters by Taguchi Orthogonal Array (TOA) Methodology and Analysis of the experimental data:

Biodegradation of pollutants is a function of various biological, physical, and chemical parameters. Taguchi's DOE approach involves determining diverse experimental conditions via orthogonal arrays (OA) to eliminate errors or noise, leading to improved efficacy and reproducibility of the bioprocess[24]. Taguchi methodology involves four different steps, which are summarized in Fig. 1. Each of these steps has a particular objective that is linked sequentially to maximize the output. In the present study, five different parameters were selected based on previous experiments owing to their crucial influence on the degradation of 4-CP biodegradation. Table 1 shows the list of five factors and their three levels chosen for this experimental study. Qualitek-4 software was used to design the experimental runs. It is a user-friendly software that is well fortified with L-4 to L-64 orthogonal arrays where the user can select 2 to 63 parameters along with their two to four levels[25]. A L-18 OA was designed by Taguchi methodology to establish the optimal experimental conditions for improved biodegradation of 4-CP. The trial runs performed under the L-18 OA, along with the experimental

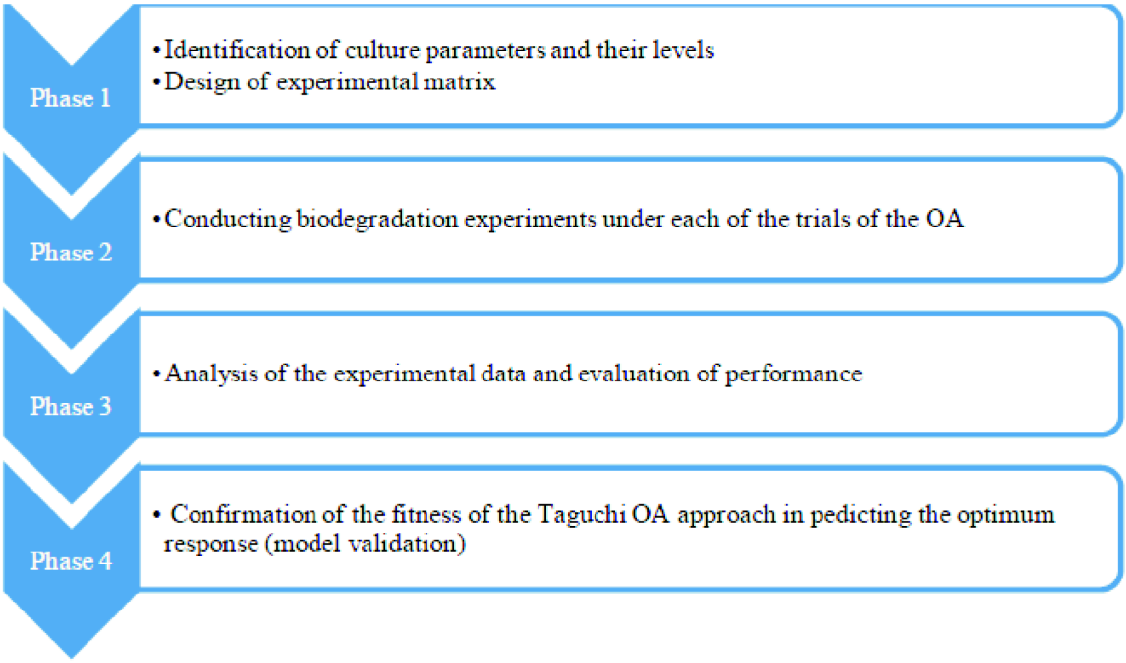

Fig. 1. Steps of the Taguchi optimization procedure[26].

Table 2. The experimental design of test runs under L-18 OA

No. of trials	A	B	C	D	E	Biomass concentration (g/L)			4-CP degradation (%)		
1.	1	1	1	1	1	0.13003	0.1246	0.12	17.64	16.86	14.65
2.	1	2	2	2	2	0.33591	0.3142	0.312	48.33	47.67	50.03
3.	1	3	3	3	3	0.30882	0.3142	0.283	43.89	43.94	44.92
4.	2	1	1	2	2	0.28173	0.2654	0.259	41.56	34.78	42.23
5.	2	2	2	3	3	0.65016	0.6176	0.595	98.5	88.59	94.00
6.	2	3	3	1	1	0.24381	0.2275	0.225	33.32	28.66	32.73
7.	3	1	2	1	3	0.30882	0.2925	0.288	47.37	42.96	48.93
8.	3	2	3	2	1	0.28173	0.2654	0.259	44.29	39.74	45.38
9.	3	3	1	3	2	0.30340	0.2871	0.283	46.34	41.09	47.74
10.	1	1	3	3	2	0.21130	0.2004	0.196	31.38	27.63	30.49
11.	1	2	1	1	3	0.31966	0.3034	0.297	47.34	41.61	48.89
12.	1	3	2	2	1	0.19504	0.1842	0.177	27.25	26.28	25.73
13.	2	1	2	3	1	0.32508	0.3088	0.302	46.39	44.04	47.8
14.	2	2	3	1	2	0.39009	0.3738	0.364	51.83	49.54	54.07
15.	2	3	1	2	3	0.39009	0.3630	0.364	50.78	48.63	52.86
16.	3	1	3	2	3	0.33591	0.3196	0.312	48.87	46.66	50.66
17.	3	2	1	3	1	0.29799	0.2871	0.283	44.7	40.88	45.85
18.	3	3	2	1	2	0.29257	0.2817	0.273	43.4	41.00	44.35

results obtained, are shown in Table 2. The process performance under the current and optimum conditions is evaluated by the Signal to Noise (S/N) ratio, which estimates the quality attributes deviating from the target output. In this study, the S/N ratio with 'the greater, the better' was selected. The S/N ratio values of the experimental output are derived from eq. (1) given below.

$$S/N = -10 \log_{10}\left(\frac{1}{n}\sum_{i=1}^{n}\frac{1}{y_i^2}\right) \quad (1)$$

where y stands for the mean of the process output (i.e. bacterial production and 4-CP biodegradation percent), n is the number of trials of each run. Validation of the process response was done by performing the biodegradation experiments under the optimized parameters established by the Taguchi DOE.

Analytical methods:

5 ml of culture broth was sampled out after a predetermined time interval and subjected to centrifugation at 5000 rpm for 15 min in 4ºC. The biomass pellet was dried in a hot air oven at 80ºC till a constant weight is obtained and is measured as dry biomass concentration (g/L). The residual 4-CP determination was done by spectrophotometric analysis of the supernatant at 280 nm[13].

Results and discussion

Optimization by Taguchi methodology and role of individual factors:

Evaluation of the experimental data obtained in Table 2 indicates that the process performance strictly depends on the culture conditions. Table 3 exhibits the levels of the independent factors that would maximize the process response. The impact of individual factors is ascertained by the magnitude of the difference of (L2-L1) output. The greater the magnitude, the higher is the effect[28]. For both 4-CP biodegradation and biomass growth, temperature was observed to impose the maximum individual effect, followed by pH, incubation duration, agitation, and inoculum size. Temperature is the most influential parameter that affects the microorganisms' metabolic activity and the structure of the catabolic enzymes indispensable for the degradation process[29]. pH influences the solubility and bioavailability of the pollutant[30]. It also affects the enzyme activity, consequently influencing the degradation rate. The influence of incubation duration is also prominent as more the duration of the biochemical reaction, the greater is the biomass growth leading to a more significant percentage removal of the target pollutant. Agitation ensures a uniform oxygen distribution and homogenous mixing of reactants within the reaction system, leading to enhanced pollutants degradation[24]. However, increasing the agitation might impede the biodegradation process owing to the induction of shear stress on the biomass[13]. Higher inoculum dose endows the microorganisms with better tolerance to the toxic load consequently leading to better survival and greater degradation of the pollutant[27].

Analysis of variance (ANOVA):

Analysis of variance (ANOVA) (Table 4) determines the degree of dependence of the process in terms of the contribution by individual control parameters. Analysis of experimental data using ANOVA helps to establish the statistically significant factors (at 95% confidence level) with their optimum levels. Based on the contribution percent revealed in ANOVA, it is evident that for both biomass production and 4-CP biodegradation, the incubation time was found to have the maximum contribution, followed by temperature, pH, inoculum size, and agitation speed. The difference in factor contribution from Table 3 and Table 4 can be attributed to the individual and interactive effects of the parameters. The observations made it evident that both microbial growth and 4-CP degradation are highly dependent on the mutual inter-

Table 3. Individual effects of the factors determined from the magnitude of L2-L1 values

	Biomass concentration (g/L)				4-CP biodegradation (%)			
Factors	Level-1	Level-2	Level-3	L2-L1	Level-1	Level-2	Level-3	L2-L1
Temperature	–12.863	–9.262	–10.735	3.6	30.321	33.798	33.024	3.3447
pH	–12.319	–9.183	–11.359	3.136	30.964	34.342	31.837	3.378
Agitation	–11.641	–10.076	–11.143	1.564	31.522	33.461	32.16	1.939
Inoculum dose	–11.852	–10.983	–10.025	0.868	31.252	32.415	33.475	1.163
Incubation time	–12.958	–10.915	–8.987	2.043	30.211	32.493	34.439	2.282

Table 4. ANOVA for biomass growth and 4-CP degradation

Factors	Biomass concentration (g/L)						4-CP degradation (%)					
	DOF (f)	Sum of squares (S)	Variance (V)	F-Ratio (F)	Pure sum (S″)	Percent (P%)	DOF (f)	Sum of squares (S)	Variance (V)	F-Ratio (F)	Pure sum (S″)	Percent (P%)
Temperature	2	39.342	19.671	75.578	38.822	28.299	2	39.982	19.991	46.519	39.122	24.422
pH	2	30.987	15.493	59.528	30.467	22.209	2	36.908	18.454	42.942	36.048	22.503
Agitation	2	7.678	3.839	14.75	7.157	5.217	2	11.718	5.859	13.633	10.858	6.778
Inoculum dose	2	10.023	5.011	19.256	9.503	6.927	2	14.837	7.418	17.262	13.977	8.725
Incubation time	2	47.327	23.663	90.918	46.807	34.12	2	53.737	26.868	62.522	52.877	33.009
Error	7	1.821	26			3.228	7	3.008	0.429			4.563
Total	17	137.182				100.00%		160.19				100.00%

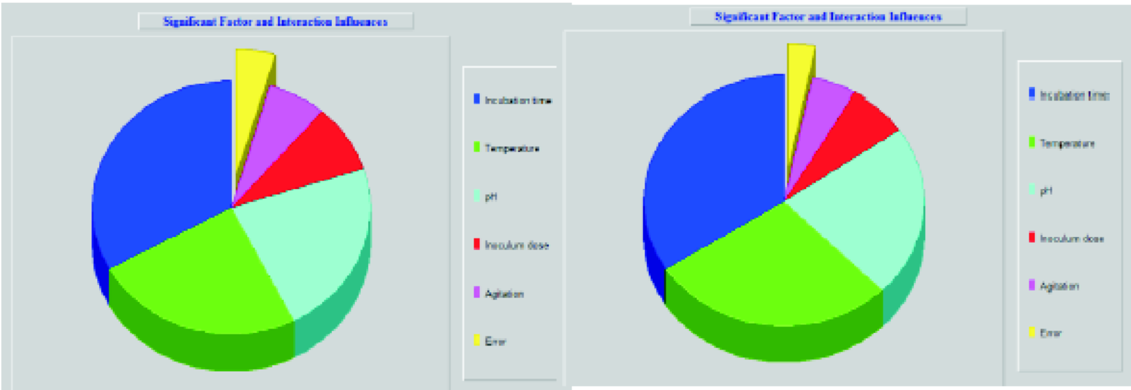

Fig. 2. Pie chart indicating the significant factors influencing (a) the biomass growth and (b) 4-CP biodegradation.

play among the factors instead of their individual influence. Fig. 2 is the pictorial representation of the influence of the significant factors on the process response.

Establishment of optimum levels of the parameters:

Table 5 shows the optimum process conditions needed for biomass generation and 4-CP biodegradation, predicted

Table 5. Optimum conditions predicted by Taguchi methodology

Coded factor	Factor	Values	Level	Contribution for biomass production from S/N ratio	Contribution for 4-CP biodegradation from S/N ratio
A	Temperature (°C)	32	2	1.691	1.417
B	pH	7.5	2	1.77	1.961
C	Agitation (RPM)	140	2	0.877	1.079
D	Inoculum dose (OD_{600})	0.6	3	0.928	1.094
E	Incubation time (h)	72	3	1.966	2.058
Total contribution from all factors				7.232	7.608
Current grand average performance				–10.953	32.381
Expected result at optimum condition				–3.721	39.99
Expected performance calculated from S/N ratio				0.652 g/L	99.885%

by the Taguchi method. The influence of the parameters on the culture growth and 4-CP biodegradation are ascertained from their absolute S/N values. The established optimum values in order of their influence on biomass proliferation and 4-CP degradation are 72 h incubation time, 7.5 pH, 32°C temperature, 0.6 OD_{600}, and 140 rpm agitation. According to the predicted values by Taguchi methodology, the expected biomass concentration, and 4-CP biodegradation percent at the optimum conditions are found to be 0.652 g/L and 99.885%, respectively.

Validation experimentation:

Confirmatory biodegradation experiments are performed at the optimum conditions established by Taguchi orthogonal design to ascertain the methodology's efficacy and reliability. Validatory experiments indicated that 0.587 g/L of biomass concentration and 99.16% 4-CP degradation percent are obtained, which are in close agreement with the predicted value.

Conclusion

The isolated consortium could utilize 500 mg/L of 4-CP as a sole carbon and energy source. Taguchi OA was successfully implemented for the optimization of control parameters for the improved biodegradation of 4-CP. The optimum conditions established by Taguchi DOE in order of their significance are 72 h incubation time, 7.5 pH, 32°C temperature, 0.6 OD_{600} inoculum size, and 140 rpm agitation. Biodegradation experiments under the established optimum conditions resulted in 0.587 g/L of biomass growth and 99.16% of 4-CP biodegradation, leading to an overall enhancement of 107.42% and 138.42%, respectively. Thus, Taguchi OA could be utilized as a practical process optimization tool and can be applied in the bioremediation of 4-CP containing industrial effluents, leading to its eco-benign disposal.

Acknowledgement

The authors sincerely thank the Department of Biotechnology (DBT), Ministry of Science and Technology, Goverment of India, for providing financial support in the form of a DBT-JRF scholarship.

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