International Journal of Environmental Science and Technology (2019) 16:5807–5816
https://doi.org/10.1007/s13762-018-1958-6

ORIGINAL PAPER

Evaluation of butachlor biodegradation efficacy of *Serratia ureilytica* strain AS1: a statistical optimization approach

S. S. Mohanty[1] · H. M. Jena[1]

Received: 13 July 2017 / Revised: 20 July 2018 / Accepted: 6 August 2018 / Published online: 13 August 2018

Abstract

Butachlor is a chloroacetanilide class of herbicide, commonly administered to control unwanted grasses and broad leaf weeds. Extensive usage of the herbicide has led to the contamination of water bodies and surrounding areas, resulting in an adverse impact on the environment. In the present work, a novel butachlor-catabolizing bacterium *Serratia ureilytica* strain AS-1 was isolated from an herbicide-contaminated soil. Statistical optimization techniques were used to optimize the butachlor biodegradation. Experimental parameters such as growth temperature, pH of the medium and biomass concentration were found to be significant for butachlor biodegradation. The results obtained indicates that the maximum degradation of 2.08 mg/L/h of butachlor was achieved under the optimal conditions of 32.5 °C of incubation temperature, pH 7.5 and 10% (v/v) inoculum size along with a polynomial mathematical model having $R^2 = 0.9833$. The model was corroborated by carrying out experiments at the optimized conditions.

Keywords Biodegradation · Butachlor · Environmental pollution · Response surface methodology · Serratia

Introduction

Chloroacetanilide is an important class of systemic selective herbicides used worldwide for controlling both pre-emergent and early post-emergent weeds in several crops such as corn, rice and soybean. (Dwivedi et al. 2012). These herbicides inhibit the synthesis of proteins, lipids, alcohols, flavonoids, lignin, etc. (Seok et al. 2012). Butachlor, a chloroacetanilide class of herbicide, is one of the most extensively recommended class of chloroacetanilide class of herbicide for controlling pre-emergent broadleaf weeds, annual grasses and submerged macrophytes in freshwater water bodies. In addition to the lipid biosynthesis, this compound also disturbs several other important metabolic pathways and redox homoeostasis negatively (Götz and Böger 2004). It is one of the most abundantly used herbicides in Asia, Africa and South America. It has been reported that yearly butachlor consumption in Asia alone is more than 4.5×10^7 kg (Ateeq et al. 2002). Extensive usage of butachlor resulted in deleterious effects on the environment. Higher concentration of butachlor residues, as well as its intermediate degradation products, has been identified in various soils as well as ground and surface waters (Kim et al. 2013). Several studies pertaining to the pernicious effect of the herbicide in the environment have been undertaken (Wang et al. 2007; Fang et al. 2009; Abigail et al. 2015). Research suggests that butachlor is known to induce apoptosis in mammalian cells and exerts genotoxic effects on amphibians (Geng et al. 2005). It is also reported to exhibit toxicity to earthworms and impact microbial community structures and enzymatic activities (Muthukaruppan et al. 2004). Various in vitro studies have established the mutagenic property of butachlor towards aquatic organisms such as freshwater fish, *Tilapia zillii* (Nwani et al. 2013) and *Salmonella typhimurium* (Hsu et al. 2005) leading to the impairment of the water environment. Moreover, some studies also reported probable carcinogenicity and oxidative DNA damage in human cells due to butachlor (Dwivedi et al. 2012).

Owing to the persistent nature of the butachlor in the agricultural soil and the threats to the environment, great interest and concernment have been invoked about the behaviour and efficient remediation of the herbicide and its metabolites in the ecosystem (Debnath et al. 2002; Yu et al. 2003; Fang

Editorial responsibility: M. Abbaspour.

✉ H. M. Jena
hara.jena@gmail.com

[1] Department of Chemical Engineering, National Institute of Technology, Rourkela 769008, India

et al. 2009). Several studies have reported that butachlor could be removed from the environment by both biotic and abiotic processes (Pal et al. 2006). However, the chemical hydrolysis and photo-oxidation of the herbicide are relatively insignificant to its removal since these are time-consuming procedures and they involve other issues such as high cost, secondary effluent problems. Hence, microbial transformation is one of the most prodigious route for determining the fate of butachlor and its dissipation from the ecosystem (Zhang et al. 2011; Rajasankar et al. 2013). In the recent years, microbial degradation has received recognition as an effective, economical and dependable alternative for remediation of various pesticide contaminations. Earlier, a few microorganisms have been reported that have the capability to use butachlor as the sole carbon and energy source (Torra-Reventos et al. 2004; Dwivedi et al. 2010). A couple of studies on the metabolic pathway and enzyme studies have also been carried out (Zhang et al. 2011; Liu et al. 2012; Gao et al. 2015). However, previously isolated microorganisms are not competent enough regarding degradation potential in most of these studies. Nevertheless, it is essential to isolate new microbes with higher butachlor removal efficiency.

Environmental parameters play an exceptionally significant factor in determining the biodegradation efficiency of any microorganism. Their degradation and cell growth potential are highly affected by both environmental and nutritional parameters as in temperature, pH, nitrogen and carbon sources (Zhou et al. 2011; Kong et al. 2014). However, as per the available literature, no study regarding the nutritional and environmental parameters affecting butachlor biodegradation exists. Hence, it is highly essential to sketch an appropriate model design for enhancing the butachlor removal efficiency by the microbe. Optimized factors improve the biodegradation efficiency and reduces the process time and cost significantly. Considering the several parameters that vary simultaneously, statistical models like response surface methodology (RSM) and Plackett–Burman designs (PBD) can optimize the concerning parameters simultaneously to dismiss the drawbacks of one factor at a time technique (Singh et al. 2017). Although several studies regarding statistical modelling and optimization of biodegradation of many toxic pollutants exist in the literature, application of mathematical optimization for the biodegradation of butachlor is yet to be studied. RSM, endorsed by a software, is a sensible approach to estimate the relationship of an array of controlled and influencing parameters. Hence, it is highly essential to draft a strategy to enhance degradation of butachlor by the isolated microbial strain.

In the present study, a butachlor-catabolizing bacterium was isolated from the agricultural soil. Statistical optimization techniques such as Plackett–Burman design and RSM were being engaged in defining the significant factors affecting butachlor biodegradation and identifying the optimum levels of those variables for maximizing the butachlor degradation by the isolated strain.

Materials and methods

Chemicals and medium

Butachlor (purity = 90.5%) from Insecticides India, Ltd., was used in this study. The rest of the chemicals and reagents were obtained from Merck (India) of the highest analytical reagent grade. The composition of the mineral salts medium (MSM) used in this study is as follows: $(NH_4)_2SO_4$ (1.0 g/L), NaCl (1.0 g/L), K_2HPO_4 (1.5 g/L), KH_2PO_4 (0.5 g/L) and $MgSO_4{\cdot}7H_2O$ (0.2 g/L) (pH 7.0) (Liu et al. 2012).

Isolation and identification of butachlor-catabolizing microorganism

The soil samples were obtained from an agricultural field in Odisha, India (20.266°N, 86.166°E) having a history of butachlor application for many years (DAFP ODISHA 2008). The enrichment culture for the isolation of butachlor-catabolizing microorganism was carried through as reported by Mohanty and Jena (2017). About five grams of the contaminated soil sample was added to 100 mL MSM comprising butachlor (100 mg/L), and the setup was incubated for five days at 35 °C, 180 RPM in an orbital shaker incubator. Then, five millilitre of the enrichment culture suspension was aseptically transferred into fresh flask containing MSM and further incubated for five more days. The concentration of butachlor was determined after each transfer to confirm its degradation. After sixth transfer, the enrichment culture was subjected to serial dilution and was transferred to MSM agar plates comprising 100 mg/L of butachlor. The pure culture obtained was evaluated for their ability to degrade butachlor. Eventually, the bacterial strains showing maximum butachlor tolerance and highest degradation potential were chosen for further studies.

For the identification of the microbial strains, 16 s rRNA gene sequences were obtained and were homologized using BLAST algorithm with the archived 16 s rDNA sequences already submitted at GeneBank, NCBI. A phylogenetic tree is developed as presented in Fig. 1 using MEGA 6.0.5 software (Thompson et al. 1997). The partial 16 s rDNA gene sequences obtained were submitted to GeneBank, NCBI.

Analytical method

The butachlor residue in the medium was estimated by HPLC analysis of the culture medium in regular interval of time. The HPLC analysis was carried out with 5-µm, C-18 column (Agilent Technologies, USA). The mixture of

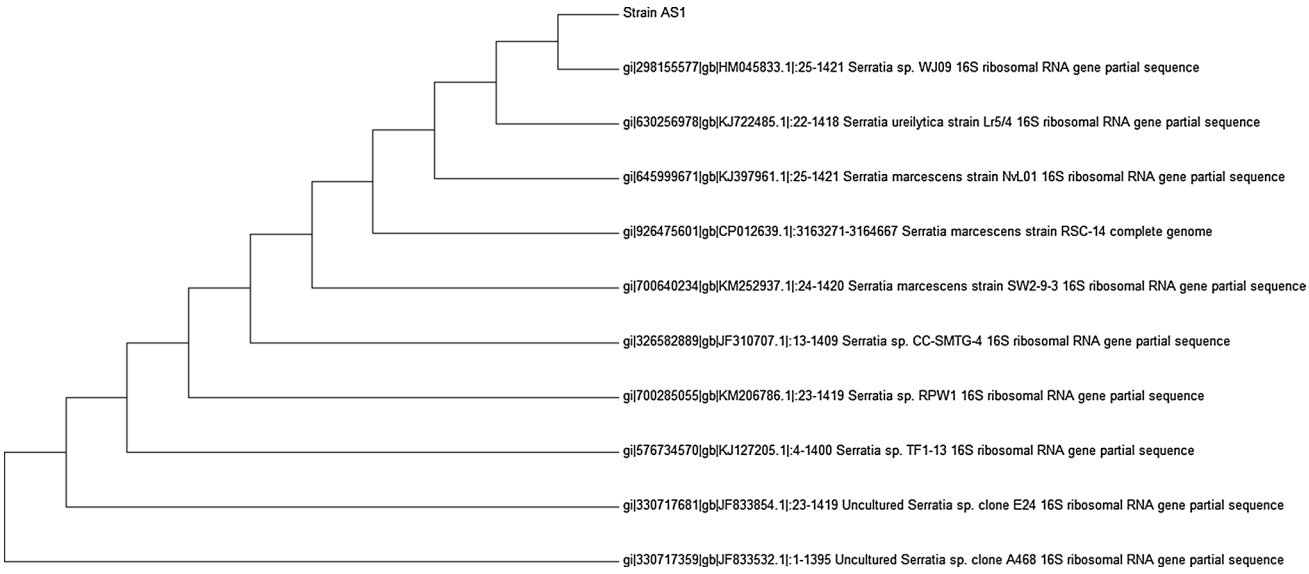

Fig. 1 Phylogenetic tree based on the 16S rRNA gene sequences of strain AS1 and related species. The GenBank accession number for each microorganism used in the analysis is shown along with the species name

methanol and water (both HPLC Grade) in a ratio of 70: 30 was asserted as the gradient mobile phase. While the flow rate was held at 1 ml/min, butachlor was quantified at wavelength of 225 nm (Dwivedi et al. 2010) (Liu et al. 2012). Each reading was performed in triplicates.

Design of experiments

Plackett–Burman design

In the present investigation, a 16-run Plackett–Burman design (including four centre points) was employed for seven variables (along with two dummy variables). The factors were been evaluated both for higher level denoted by +1 and lower level denoted by −1 while the centre value was denoted as level zero. The boundary value as well as the value of the centre point of variable has been defined as per the results obtained in preliminary studies (data not shown here). The parameters considered for the study, their corresponding −1 and +1 values, the design and the corresponding response (butachlor degradation) values have been enlisted in Table 1. The effect of each parameter on butachlor degradation has been calculated using the following equation

$$Y = M_0 + \Sigma M_i X_i \quad (1)$$

where Y denotes the response (percentage butachlor removal), M_0: model intercept; M_i: linear factor coefficient; X_i: Participant variable (Dayana Priyadharshini and Bakthavatsalam 2016). The factors having p value less than 5% in the regression analysis were considered to have a crucial effect on butachlor biodegradation and were studied further by RSM.

Response surface methodology

Utilizing the Plackett–Burman analysis, three influential factors (pH of the medium, growth temperature and biomass concentration) imperative for biodegradation of butachlor were shortlisted. To optimize the significant factors for the biodegradation of butachlor, a 2^3 full factorial central composite design (CCD), each at five levels ($-\alpha$, −1, 0, +1, $+\alpha$) with six replicates at the centre points and eight axial points, was employed for improving butachlor degradation by the isolated strain. An altogether of 20 experiments was carried out, and the particulars of the experimental design have been enlisted in Table 2. A second-order polynomial equation was engaged for analysing the experimental data obtained and calculates the relationship between the studied parameters as shown by Eq. (2):

$$\text{Response} = M_0 + \sum_{i=1}^{n} M_i X_i + \sum_{i=1}^{n} M_{ii} X_i^2 + \sum_{i=1}^{n} \sum_{j=1}^{n} M_{ij} X_i X_j \quad (2)$$

wherein response denotes the predicted percentage of butachlor removal; X symbolizes the input variables influencing the response; M_0 is the constant (intercept coefficient); M_i represents the ith linear coefficient; M_{ii} intends to be ith quadratic coefficient, and M_{ij} stands for the ijth interaction coefficient (Zhao et al. 2017). For establishing the interaction among the variables, surface plots (three-dimensional) of the predicted responses were constructed. The statistical

Table 1 Plackett–Burman design of factors with butachlor degradation (percentage) as a response

Run order	X_1 Temperature (°C)	X_2 pH	X_3 Inoculum Size (%)	X_4 $(NH_4)_2SO_4$ (g/100 ml)	X_5 KH_2PO_4 (g/100 ml)	X_6 K_2HPO_4 (g/100 ml)	X_7 NaCl (g/100 ml)	Experimental value	Predicted value
1	25	6	15	0.2	0.125	0.075	0.2	43.9	43.001658
2	40	6	15	0.075	0.05	0.075	0.2	51.5	49.829995
3	40	6	5	0.075	0.125	0.2	0.2	51.8	54.674998
4	40	6	15	0.2	0.05	0.2	0.075	52.04	52.62167
5	25	6	5	0.2	0.125	0.2	0.075	48.52	46.849995
6	40	9	5	0.2	0.125	0.075	0.2	53.15	52.843318
7	32.5	7.5	10	0.1375	0.0875	0.1375	0.1375	70.23	70.639155
8	32.5	7.5	10	0.1375	0.0875	0.1375	0.1375	72.51	70.639155
9	40	9	5	0.2	0.05	0.075	0.075	51.54	51.846653
10	32.5	7.5	10	0.1375	0.0875	0.1375	0.1375	75.69	70.639155
11	25	9	5	0.075	0.05	0.2	0.2	37.96	35.973313
12	40	9	15	0.075	0.125	0.2	0.075	48.12	46.333318
13	25	9	15	0.075	0.125	0.075	0.075	33.93	35.71664
14	32.5	7.5	10	0.1375	0.0875	0.1375	0.1375	72.62	70.639155
15	25	6	5	0.075	0.05	0.075	0.075	42.28	43.061655
16	25	9	15	0.2	0.05	0.2	0.2	32.93	34.91665

Table 2 Effect of factors and statistical analysis of factors using Plackett–Burman Design

	Effect	Standard error	*T* value	*P* value
Term constant		0.7330	62.26	0.000
X_1	11.438	0.7330	7.80	0.000
X_2	−5.402	0.7330	−3.68	0.008
X_3	−3.805	0.7330	−2.60	0.036
X_4	2.748	0.7330	1.87	0.103
X_5	1.862	0.7330	1.27	0.245
X_6	−0.822	0.7330	−0.56	0.593
X_7	−0.865	0.7330	−0.59	0.574

software Minitab (Version 17.1) has been used for designing the experiment and further determination of the regression coefficients by analysis of variance (ANOVA) and the coefficient of determination (R^2).

Results and discussion

Isolation and characterization of butachlor-catabolizing strain

From the preliminary screening of 12 bacterial isolates obtained from the enrichment culture, three bacterial strains designated AS1, AS2, and AS5 were initially selected for the study taking into account their high butachlor tolerance potential. Among the isolates, the bacterial strain-AS1 was capable of tolerating butachlor concentration as high as 1000 mg/L and was able to degrade up to 100 mg/L of butachlor within 48 h. Hence, the strain was selected for further investigation. The strain-AS1 is a Gram-negative, motile, rod-shaped, non-spore forming bacterium. The microbial strain has been positive for starch hydrolysis, urease, Voges–Proskauer and nitrate reduction test while negative for oxidase and lactose hydrolysis. The 16S rDNA sequence obtained has been submitted in the GenBank repository with Accession No. KT427634. The multiple sequence alignment of the obtained sequence and the sequences available in GenBank archives revealed the highest degree of similarity with the members of the genus Serratia and forming a subclade with *S. ureilytica* KJ722485 (100% homology) (Fig. 1). Thus, because of the aspects stated above, the strain-AS1 was identified as *S. ureilytica*.

Optimization of biodegradation of butachlor by *Serratia ureilytica*

The seven parameters contemplated in this study for their influence on biodegradation of butachlor by the microbial strain were statistically analysed using PBD. Table 1 enlists the design of experiments and their corresponding butachlor degradation percentage. The wide variation in responses from 32.93 to 75.69, in the 16 trials, emphasizes the dependency of butachlor removal efficiency of the microbe on various process parameters. Table 3 exhibited that parameters such as pH of the medium, growth temperature and inoculum size have a substantial impact on the biodegradation of butachlor and were hence incorporated in the subsequent

Table 3 ANOVA for PBD (Plackett–Burman Design) model

Source	DF	Seq SS	Adj SS	Adj MS	*F*	*P*
Main effects	7	560.80	560.80	80.11	12.43	0.002
X_1	1	392.51	392.51	392.51	60.88	0.000
X_2	1	87.53	87.53	87.53	13.58	0.008
X_3	1	43.43	43.43	43.43	6.74	0.036
X_4	1	22.66	22.66	22.66	3.51	0.103
X_5	1	10.40	10.40	10.40	1.61	0.245
X_6	1	2.03	2.03	2.03	0.31	0.593
X_7	1	2.24	2.24	2.24	0.35	0.574
Curvature	1	2207.03	2207.03	2207.03	342.30	0.000
Residual error	7	45.13	45.13	6.45	1.50	**0.386**
Lack of fit	4	30.07	30.07	7.52		
Pure error	3	15.07	15.07	5.02		
Total	15	2812.96				

The lack of fit for each statistical design (both PBD and CCD) has been written in bold to signify its importance. Lack of fit is an important parameter for any statistical design and it determines the validity of any mathematical model. The lack of fit of any model should be insignificant to establish the competence of the model

optimization study. However, the rest of the parameters had no significant effect on biodegradation of butachlor. The factors are having a confidence level more than 95% were considered as important for their incorporation in the subsequent studies of optimization. The model equation for the percentage butachlor biodegradation (Y):

$$Y = 34.8255 + 0.762556X_1 - 1.80056X_2 - 0.380500X_3 + 21.9867X_4 + 24.8222X_5 - 6.5733X_6 - 6.9200X_7 \quad (3)$$

The correlation coefficient (R^2) of the value 0.984 suggested that up to 98.4% variabilities in the butachlor degradation could be calculated. The statistical analysis of the experimental data using F-test is presented in Table 4.

The CCD was put to use for determining the interactions among the significant parameters viz. Growth temperature (X_1), pH of the medium (X_2) and biomass concentration (X_3) for further optimization studies. Table 2 showcases the experimental design matrix and the corresponding results obtained. The following second-order polynomial equation has been deduced after the application of multiple regression analysis for the analysis of the experimental data:

$$Y = 75.146 + 5.806X_1 - 1.611X_2 + 8.802X_3 - 7.329X_1^2 - 11.803X_2^2 - 4.520X_3^2 \quad (4)$$

where Y denotes the percentage butachlor degradation. Table 5 presents the ANOVA of the proposed model for the percentage butachlor degradation. The significance of the model was suggested by its "F value" which was found to be 127.79. The linear as well as the quadratic terms were significant for the estimation of butachlor biodegradation efficiency. Both the experimental and the predicted response are in good agreement with each other as suggested by the R^2 value of 0.9833. The "Lack of Fit" of 0.067 suggested it to be non-significant compared to the pure error which means only 6.7% probability of occurrence of "Lack of Fit" due to

Table 4 Experimental design and results of CCD

Run order	X_1 Temperature (°C)	X_2 pH	X_3 Inoculum size (%)	Response	Predicted value
1	0	0	0	75.23	75.1473
2	−α	0	0	40.16	44.65307
3	−1	1	−1	38.675	35.27589
4	−1	1	1	54.79	52.88009
5	0	+α	0	36.48	39.05329
6	−1	−1	1	59.21	56.10134
7	0	0	−α	45.72	47.55872
8	1	−1	1	68.98	67.71269
9	0	0	0	74.96	75.1473
10	0	−α	0	43.29	44.47076
11	1	1	1	63.57	64.49143
12	0	0	0	75.37	75.1473
13	0	0	+α	75.25	77.16534
14	−1	−1	−1	39.78	38.49714
15	0	0	0	75.42	75.1473
16	+α	0	0	64.92	64.18096
17	1	1	−1	48.97	46.88723
18	0	0	0	75.12	75.1473
19	0	0	0	75.42	75.1473
20	1	−1	−1	48.58	50.10849

Table 5 ANOVA for CCD model

Source	DF	Seq SS	Adj SS	Adj MS	*F*	*P*
Regression	6	4227.83	4227.83	704.64	7.79	0.000
Linear	3	1553.82	1553.82	517.94	3.93	0.000
X_1	1	460.30	460.30	460.30	83.48	0.000
X_2	1	35.43	35.43	35.43	6.43	0.025
X_3	1	1058.08	1058.08	1058.08	191.89	0.000
Square	3	2674.01	2674.01	891.34	161.65	0.000
X_1^2	1	503.03	774.15	774.15	140.40	0.000
X_2^2	1	1876.52	2007.81	2007.81	364.14	0.000
X_3^2	1	294.47	294.47	294.47	53.40	0.000
Residual error	13	71.68	71.68	5.51		
Lack of fit	8	71.51	71.51	8.94	257.54	**0.067**
Pure error	5	0.17			0.17	0.03
Total	19	4299.51				

Lack of fit is an important parameter for any statistical design and it determines the validity of anymathematical model. The lack of fit of any model should be insignificant to establish the competence of themodel

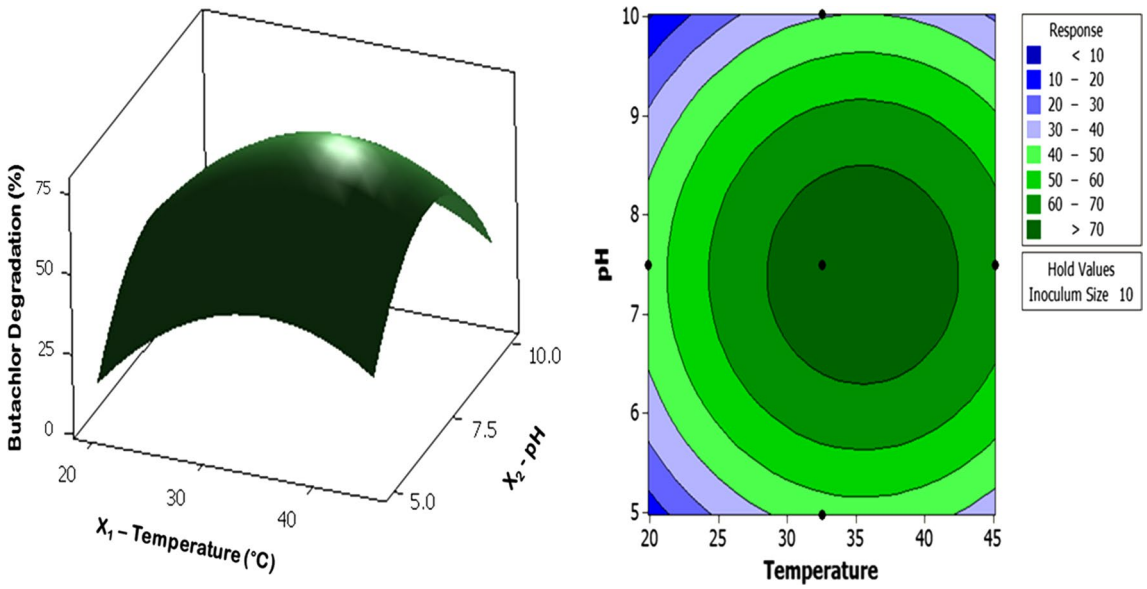

Fig. 2 Surface and contour plot showing interactions between temperature (X_1) and pH (X_2) on butachlor biodegradation by the isolated *S. ureilytica* strain AS1 while inoculum size (X_3) at zero level

noise. The established model is sufficiently competent to predict the biodegradation of butachlor within the ranges of the dedicated variables.

The regression model was graphically represented by surface plots (three-dimensional) and corresponding contour plots (two-dimensional) (Figs. 2, 3, 4) which were used to contemplate the effect of each parameter individually as well as their mutual interaction among themselves, on biodegradation of butachlor. The plots are based on Eq. (4), where holding one variable at its optimal level, the effect of the other two variables was studied by varying them within the experimental boundaries, the pattern of which indicates the significance of the mutual interaction among the independent variables. As demonstrated in the figures, a clear peak as response surface for butachlor degradation meant that the optimum points are well within the design boundaries.

Figure 2 depicts the effect of growth temperature (X_1) versus pH of the medium (X_2) on butachlor biodegradation keeping the initial inoculum size (X_3) constant at level zero. The response surface plot indicates that change in the growth temperature as well as the in surrounding pH has a significant impact on the degradation efficiency of the microbial strain. The butachlor removal efficiency increases with increasing temperature, but after a certain point, the

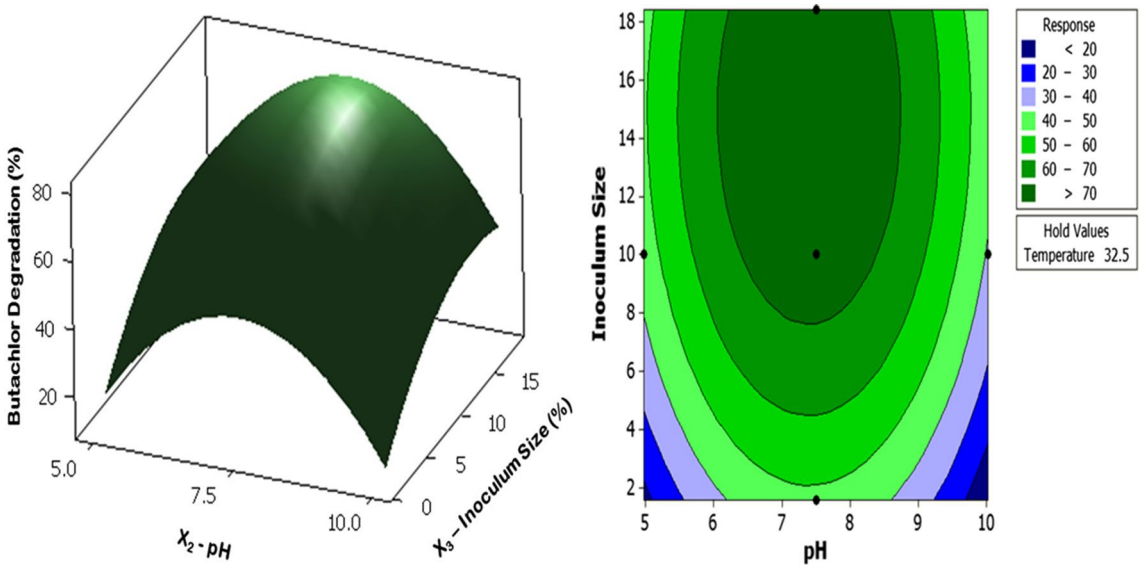

Fig. 3 Surface and contour plot showing interactions between pH (X_2) and inoculum size (X_3) on butachlor biodegradation by the isolated *S. ureilytica* strain AS1 while temperature (X_1) at zero level

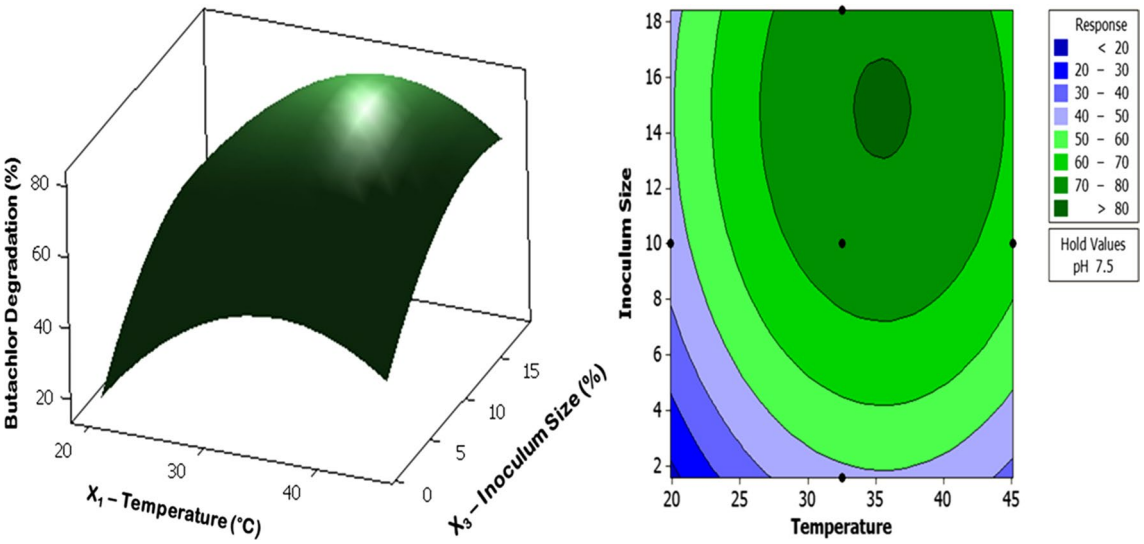

Fig. 4 Surface and contour plot showing interactions between temperature (X_1) and inoculum size (X_3) on butachlor biodegradation by the isolated *S. ureilytica* strain AS1 while pH (X_2) at zero level

biodegradation efficiency declines on further increase in the temperature. A similar pattern was observed for the change in the surrounding pH. The uniformly elongated diagonal pattern of the contour plots suggested the significance of the interaction between temperature and pH on butachlor biodegradation. Similar results were reported during the biodegradation of profenofos by a novel bacterial consortium (Jabeen et al. 2015).

The influence of pH of the medium (X_2) and the inoculum size of the microbial strain (X_3) on butachlor biodegradation keeping the temperature constant has been depicted in Fig. 3. The figure suggests that the optimum butachlor degradation can be attained by keeping the surrounding pH value within 7–8 and increasing the concentration of the microbial inoculum for the study. The elongated two-dimensional contour plot of butachlor degradation versus pH, inoculum size suggests that the mutual interaction among the parameters is significant to the butachlor biodegradation by the isolated strain. Similar results were suggested in the previous study of biodegradation of phenol by the *Chlorella pyrenoidosa*

where the concentration of the biomass in the medium plays a major role in the biodegradation of phenol (Dayana Priyadharshini and Bakthavatsalam 2016).

The plot representing the significance of growth temperature (X_1) and the inoculum size (X_3) on biodegradation of butachlor while keeping the pH of the medium (X_2) at zero level has been depicted in Fig. 4. As presented in the figure, with an increase in the concentration of the biomass and the temperature, the butachlor biodegradation increases. However, the degradation efficiency decreases with increasing the initial biomass concentration as well as the incubation temperature further beyond the optimal conditions. The elongated running two-dimensional contour plot suggests that at a particular pH, the growth temperature and the initial biomass concentration are interdependent on each other and this relationship is vital for the biodegradation of butachlor by the microbial strain. This is in agreement with the earlier works where inoculum size portrays a major role in the biodegradation of the organic contaminant (Zhou et al. 2011).

To validate the obtained statistical model and propose a better understanding of the biodegradation of butachlor, triplicate experiments were performed at optimum levels of the independent variables. The predicted butachlor removal efficiency under the optimal conditions was 79.42% against the actual experimental removal efficiency of 81.08%. Since the predicted and the actual measured values were found to be very close, the validity of the predicted RSM model was thus substantiated.

Biodegradation of butachlor by the strain AS1

Figure 5 demonstrates the biodegradation of butachlor by the isolated *S. ureilytica* strain AS1 under the optimized condition. The figure exhibits the gradual decrease in the butachlor concentration as a function of time. HPLC analysis quantifies the concentration of the butachlor at a particular time point. Significant reduction in the major butachlor peak at the retention time of 14 min along with the subsequent emergence of numerous secondary peaks of unknown metabolites has been observed at different time points (data not presented). The microbial *S. ureilytica* strain AS1 displayed complete butachlor degradation of 500 mg/L of butachlor within 10 days at optimized conditions. Increasing the concentration of the butachlor in the medium resulted in the inhibition of the degradation efficiency of the bacterial strain. A prolonged lag phase observed in MSM comprising more than 500 mg/L is attributed to the adaptation phase, possibly owing to the fact that the microorganism takes time to process signal transduction and subsequently induce a metabolic pathway for biodegradation. Similar results have been observed in previous studies reporting prolonged lag phase in similar intensified conditions (Dwivedi et al. 2010; Mohanty 2012).

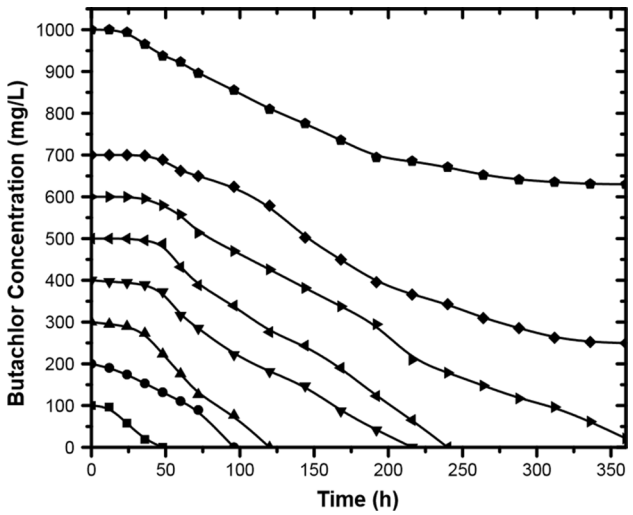

Fig. 5 Degradation profile of the isolate *S. ureilytica* strain AS1 at different initial concentration of butachlor

Discussion

Biological treatment methods have been proved to be promising alternatives to remediate contaminated environments. In this study, twelve bacterial strains were isolated from soil samples obtained from an agricultural field with a history of application of the herbicide. Using the selective enrichment technique, an efficient butachlor-catabolizing strain AS 1 has been isolated. The microbial strains were assessed for their butachlor tolerance ability within the range of 100–300 mg/L of butachlor. The prime focus of the study was to isolate and identify the major bio-remediating microbial agents and assess their butachlor remediation capability in the optimized conditions. Based on the comparative analysis of the phenotypic attributes of the isolated strain AS1 and its phylogenetic analysis revealed to form a subclade with similarity with *S. ureilytica* sp. nov. KJ722485 (100% similarity) (Bhadra et al. 2005). Strain AS 1 showed higher tolerance and better degradation characteristics from previously reported butachlor degraders. This study might be the basis for screening, production and utilization of microbial strains as bioremediation agents for commercial purposes in the future. The microorganism *Serratia* has earlier been reported to utilize few hydrocarbons for the production of the dyes and remediate some heavy metals (Pakala et al. 2007; Rahman et al. 2009; Venkateswar Reddy et al. 2015). However, to the very best of our understanding, this is the first of its kind report pertaining to the exploration of the herbicide remediation aspect of the strain *S. ureilytica*. Even though, a few microorganisms have been reported for the biodegradation of butachlor, thus far, no precise and methodological study on butachlor biodegradation has been carried

out. This study provides the first evidence that the strain AS1 has the potential in the efficient degradation of butachlor which is extensively used in the agriculture of various crops and is of global concern (Abigail et al. 2015).

The novelty of the present investigation is the isolation of a high tolerating butachlor-catabolizing microorganism and mathematical optimization of butachlor degradation by the same. Application of the statistical design of experiment for screening and optimizing various experimental parameters renders a prompt recognition of the significant factors and interaction among them. To the best of our knowledge, this is the first study to report on the application of statistical design of experiments and mathematical modelling for optimization of the process of butachlor biodegradation. Application of PBD elucidated the facts that the parameters such as pH of the medium, growth temperature and initial biomass concentration has a significant impact on the biodegradation of butachlor and was hence incorporated in the subsequent optimization study. RSM elaborated the influence of the above-mentioned parameters in a more obvious way and reported the interactive effect of different variables on butachlor biodegradation.

Even though a few microorganisms having the capability to degrade butachlor have been reported earlier, there is no report of complete degradation by any microorganism (Dwivedi et al. 2010; Kim et al. 2013). Another distinguished characteristic of this particular microorganism is its ability to withstand high concentration of butachlor of 1000 mg/L. In contrast to the previous studies pertaining to the biodegradation of butachlor or other chloroacetanilide herbicides which evaluated the biodegradation potential of the microorganism only up to 100 mg/L, this bacterial strain is capable of complete biodegradation up to 500 mg/L of butachlor within 240 h. The microorganism *S. ureilytica* strain AS1 is able to utilize the butachlor exclusively as the source of carbon and energy. However, on increasing the concentration of the herbicide, inhibition in terms of both growth and degradation potential can be observed. *Serratia ureilytica* strain AS1 proves to be a promising candidate as an accomplished butachlor degrading strain and may be utilized for the treatment of effluents with high butachlor concentration.

Conclusion

The present study is the first of its kind that presents an account of the application of statistical designs for optimizing the biodegradation of chloroacetanilide class of herbicide butachlor by the newly isolated *S. ureilytica* strain. The purpose of the study was to identify the significant parameters that affect the degradation efficiency of the microbial strain and to achieve the maximum output. Among the parameters evaluated, incubation temperature, medium pH and the initial microbial biomass were found to be the important ones that influence the biodegradation of butachlor by the microbial strain the most. Statistical methods were proven to be an effective and competent tool for elucidation of optimization of various process parameters for maximizing butachlor degradation by the isolated strain and the effect of mutual interaction among them. The bacterial *S. ureilytica* strain AS1 was established to be a highly efficient and promising candidate for the biodegradation of butachlor. The results obtained in this study laid the groundwork for the employment of this isolated microbial strains for the remediation of highly concentrated butachlor contaminated water, soil and sediments.

Acknowledgements The authors wish to thank all who assisted in conducting this work.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest regarding the subject of the manuscript.

References

Abigail MEA, Samuel SM, Ramalingam C (2015) Addressing the environmental impacts of butachlor and the available remediation strategies: a systematic review. Int J Environ Sci Technol 12:4025–4036. https://doi.org/10.1007/s13762-015-0866-2

Ateeq B, Abul farah M, Niamat Ali M, Ahmad W (2002) Induction of micronuclei and erythrocyte alterations in the catfish Clarias batrachus by 2,4-dichlorophenoxyacetic acid and butachlor. Mutat Res Genet Toxicol Environ Mutagen 518:135–144. https://doi.org/10.1016/s1383-5718(02)00075-x

Bhadra B, Roy P, Chakraborty R (2005) *Serratia ureilytica* sp. nov., a novel urea-utilizing species. Int J Syst Evol Microbiol 55:2155–2158. https://doi.org/10.1099/ijs.0.63674-0

DAFP Odisha (2008) Manual on agricultural production technology. Bhubaneswar

Dayana Priyadharshini S, Bakthavatsalam AK (2016) Optimization of phenol degradation by the microalga *Chlorella pyrenoidosa* using Plackett–Burman design and response surface methodology. Bioresour Technol 207:150–156. https://doi.org/10.1016/j.biortech.2016.01.138

Debnath A, Das AC, Mukherjee D (2002) Persistence and effect of butachlor and basalin on the activities of phosphate solubilizing microorganisms in wetland rice soil. Bull Environ Contam Toxicol 68:766–770. https://doi.org/10.1007/s001280319

Dwivedi S, Singh BR, Al-Khedhairy AA, Alarifi S, Musarrat J (2010) Isolation and characterization of butachlor-catabolizing bacterial strain Stenotrophomonas acidaminiphila JS-1 from soil and assessment of its biodegradation potential. Lett Appl Microbiol 51:54–60. https://doi.org/10.1111/j.1472-765x.2010.02854.x

Dwivedi S, Saquib Q, Al-Khedhairy AA, Musarrat J (2012) Butachlor induced dissipation of mitochondrial membrane potential, oxidative DNA damage and necrosis in human peripheral blood mononuclear cells. Toxicology 302:77–87. https://doi.org/10.1016/j.tox.2012.07.014

Fang H, Yu YL, Wang XG, Chu XQ, Yang XE (2009) Persistence of the herbicide butachlor in soil after repeated applications and its effects on soil microbial functional diversity. J Environ Sci Health B 44:123–129. https://doi.org/10.1080/10934520802539657

Gao Y, Jin L, Shi H, Chu Z (2015) Characterization of a novel butachlor biodegradation pathway and cloning of the debutoxylase (Dbo) gene responsible for debutoxylation of butachlor in bacillus sp. hys-1. J Agric Food Chem. https://doi.org/10.1021/acs.jafc.5b03326

Geng BR, Yao D, Xue QQ (2005) Acute toxicity of the pesticide Dichlorvos and the herbicide butachlor to tadpoles of four anuran species. Bull Environ Contam Toxicol 75:343–349. https://doi.org/10.1007/s00128-005-0759-z

Götz T, Böger P (2004) The very-long-chain fatty acid synthase is inhibited by chloroacetamides. Zeitschrift fur Naturforsch—Sect C J Biosci 59:549–553

Hsu K-Y, Lin H-J, Lin J-K, Kuo W-S, Ou Y-H (2005) Mutagenicity study of butachlor and its metabolites using *Salmonella typhimurium*. J Microbiol Immunol Infect 38:409–416

Jabeen H, Iqbal S, Anwar S, Parales RE (2015) Optimization of profenofos degradation by a novel bacterial consortium PBAC using response surface methodology. Int Biodeterior Biodegrad 100:89–97. https://doi.org/10.1016/j.ibiod.2015.02.022

Kim NH, Kim DU, Kim I, Ka JO (2013) Syntrophic biodegradation of butachlor by Mycobacterium sp. J7A and Sphingobium sp. J7B isolated from rice paddy soil. FEMS Microbiol Lett 344:114–120. https://doi.org/10.1111/1574-6968.12163

Kong Y, Zou P, Miao L, Qi J, Song L, Zhu L, Xu X (2014) Medium optimization for the production of anti-cyanobacterial substances by Streptomyces sp. HJC-D1 using response surface methodology. Environ Sci Pollut Res 21:5983–5990. https://doi.org/10.1007/s11356-014-2532-5

Liu HM, Cao L, Lu P, Ni H, Li YX, Yan X, Hong Q, Li SP (2012) Biodegradation of butachlor by rhodococcus sp. strain B1 and purification of its hydrolase (ChlH) responsible for *N*-dealkylation of chloroacetamide herbicides. J Agric Food Chem 60:12238–12244

Mohanty SS (2012) Microbial degradation of phenol: a comparative study. National Institute of Technology, Rourkela

Mohanty SS, Jena HM (2017) Biodegradation of phenol by free and immobilized cells of a novel Pseudomonas sp. NBM11. Braz J Chem Eng 34:75–84. https://doi.org/10.1590/0104-6632.20170341s20150388

Muthukaruppan G, Janardhanan S, Vijayalakshmi G (2004) Sublethal toxicity of the herbicide butachlor on the earthworm perionyx sansibaricus and its histological changes. J Soils Sediments 5:82–86. https://doi.org/10.1065/jss2004.09.111

Nwani CD, Ama UI, Okoh F, Oji UO, Ogbonyealu RC, Agha A, Udu-Ibiam O (2013) Acute toxicity of the chloroacetanilide herbicide butachlor and its effects on the behavior of the freshwater fish *Tilapia zillii*. Afr J Biotechnol 12:499–503. https://doi.org/10.5897/ajb12.2433

Pakala SB, Gorla P, Pinjari AB, Krovidi RK, Baru R, Yanamandra M, Merrick M, Siddavattam D (2007) Biodegradation of methyl parathion and p-nitrophenol: evidence for the presence of a p-nitrophenol 2-hydroxylase in a Gram-negative Serratia sp. strain DS001. Appl Microbiol Biotechnol 73:1452–1462. https://doi.org/10.1007/s00253-006-0595-z

Pal R, Das P, Chakrabarti K, Chakraborty A, Chowdhury A (2006) Butachlor degradation in tropical soils: effect of application rate, biotic-abiotic interactions and soil conditions. J Environ Sci Health B 41:1103–1113. https://doi.org/10.1080/03601230600851141

Rahman MFA, Shukor MY, Suhaili Z, Mustafa S, Shamaan NA, Syed MA (2009) Reduction of Mo(VI) by the bacterium Serratia sp. strain DRY5. J Environ Biol 30:65–72

Rajasankar R, Manju Gayathry G, Sathiavelu A, Ramalingam C, Saravanan VS (2013) Pesticide tolerant and phosphorus solubilizing Pseudomonas sp. strain SGRAJ09 isolated from pesticides treated Achillea clavennae rhizosphere soil. Ecotoxicology 22:707–717. https://doi.org/10.1007/s10646-013-1062-0

Seok SJ, Choi SC, Gil HW, Yang JO, Lee EY, Song HY, Hong SY (2012) Acute oral poisoning due to chloracetanilide herbicides. J Korean Med Sci 27:111–114. https://doi.org/10.3346/jkms.2012.27.2.111

Singh V, Haque S, Niwas R, Srivastava A, Pasupuleti M, Tripathi CKM (2017) Strategies for fermentation medium optimization: an in-depth review. Front Microbiol 7:2087

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25:4876–4882. https://doi.org/10.1093/nar/25.24.4876

Torra-Reventos M, Yajima M, Yamanaka S, Kodama T (2004) Degradation of the herbicides thiobencarb, butachlor and molinate by a newly isolated Aspergillus niger. J Pestic Sci 29:214–216. https://doi.org/10.1584/jpestics.29.214

Venkateswar Reddy M, Kotamraju A, Venkata Mohan S (2015) Bacterial synthesis of polyhydroxyalkanoates using dark fermentation effluents: comparison between pure and enriched mixed cultures. Eng Life Sci 15:646–654. https://doi.org/10.1002/elsc.201500016

Wang J, Lu Y, Shen G (2007) Combined effects of cadmium and butachlor on soil enzyme activities and microbial community structure. Environ Geol 51:1221–1228. https://doi.org/10.1007/s00254-006-0414-y

Yu YL, Chen YX, Luo YM, Pan XD, He YF, Wong MH (2003) Rapid degradation of butachlor in wheat rhizosphere soil. Chemosphere 50:771–774. https://doi.org/10.1016/s0045-6535(02)00218-7

Zhang J, Zheng JW, Liang B, Wang CH, Cai S, Ni YY, He J, Li SP (2011) Biodegradation of chloroacetamide herbicides by Paracoccus sp. FLY-8 in vitro. J Agric Food Chem 59:4614–4621. https://doi.org/10.1021/jf104695g

Zhao X, Wang L, Ma F, Bai S, Yang J, Qi S (2017) Pseudomonas sp. ZXY-1, a newly isolated and highly efficient atrazine-degrading bacterium, and optimization of biodegradation using response surface methodology. J Environ Sci (China) 54:152–159. https://doi.org/10.1016/j.jes.2016.06.010

Zhou J, Yu X, Ding C, Wang Z, Zhou Q, Pao H, Cai W (2011) Optimization of phenol degradation by Candida tropicalis Z-04 using Plackett-Burman design and response surface methodology. J Environ Sci 23:22–30. https://doi.org/10.1016/s1001-0742(10)60369-5