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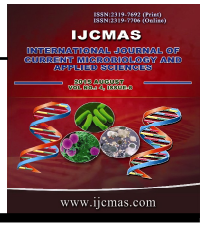

Original Research Article

Optimization of Sulfosulfuron Biodegradation through Response Surface Methodology using Indigenous Bacterial Strain Isolated from Contaminated Agriculture Field

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Keywords

Bacillus sp, Biodegradation, Sulfosulfuron, Response surface methodology

A B S T R A C T

The persistent nature of sulfosulfuron besides its less toxicity comparative to other pesticides necessitates the search for potential strains for its degradation. The soil sample was collected from the contaminated site of Udham Singh Nagar. Screening and isolation was done on the minimal media and a potential strain was selected which showed maximum degradation of sulfosulfuron. For the bioremediation of sulfosulfuron RSM (Response surface Methodology) was used. It was found that the optimum pH, Temperature and shaking speed are important for biodegradation of sulfosulfuron which was optimized by RSM. Isolated bacterial strain SA2 characterized on the basis of molecular approach it was found that it belongs to *Bacillus* sp. The report showed that the optimized conditions, pH-7 shaking speed 90 (rpm) and temperature 28^0C for maximum biodegradation (83%) of sulfosulfuron.

Introduction

Sulfosulfuron, a new sulfonylurea herbicide, is used to control weeds in wheat and other agricultural crops (Parrish *et al.*, 1995). These new herbicides collectively span a wide range of soil residual properties that are assigned to meet specific agricultural needs (Maheshwari and Ramesh, 2007). Sulfonylurea herbicides are composed of three distinct parts: an aryl group, a sulfonylurea bridge and a S-triazine group. Some reasons for their rapid acceptance include a low application rate, a very low animal toxicity and a broad-spectrum weed control. The mode of action of sulfonylurea herbicides consist in inhibiting acetolactate synthase, a key enzyme in the biosynthetic pathway of branched – chain amino acids. The three principal ways of the elimination of sulfonylurea herbicides in the environment are chemical hydrolysis, microbial degradation and photodegradation. Possible transformation in sulfonylurease are cleavage of the sulfonylurea bridge yielding sulfonamide, S-triazine and S-triazinurea bridge contraction and ring opening to form triurets (Sarmah *et al.*, 1998). Soil pH, temperature, moisture and organic matter are the major factors that influence sulfonylurea chemical hydrolysis and microbial degradation. Sulfonylurea hydrolysis in soil is mainly pH dependent and its rate increases with decreasing soil

pH (Beyer *et al.*, 1988). Latest research has proved a similar pH dependence of sulfosulfuron hydrolysis in soil incubation studies (Saha and Kulshrestha, 2002). Pusino *et al.* (2003) in their study on adsorption and desorption of a sulfonylurea herbicide triasulfuron in three soils reported that pH was the main factor influencing the adsorption and that adsorption on soils was negatively correlated with pH. The highest level of adsorption was measured on soils with low pH and high organic carbon content. Till date, there are only limited scientific reports on the sulfosulfuron biodegradation. Therefore we attempt to use the indigenous potential Bacillus sp. for biodegradation of sulfosulfuron.

Material and Methods

Chemicals and media

Technical grade sulfosulfuron was obtained from Department of Chemistry of the University. All the chemicals and solvents used in this study were of analytical grade. Sulfosulfuron was dissolved in acetonitrile to make a stock of 1mg/ml, and filter sterilized. Mimimal salt nutrient broth (pH 7.0) was used for the isolation and cultivation of sulfosulfuron degrading bacterial strain by Negi *et al.* (2014).

Screening and isolation of sulfosulfuron degrading bacteria

Pesticide contaminated soil from the agricultural fields of Udham Singh nagar Uttarakhand, India was collected and used for the isolation of sulfosulfuron degrading bacteria. One gram soil was suspended in sterile water and diluted subsequently. After dilution, soil suspension was inoculated in nutrient agar plates.

Bacterial colonies appeared after 24 hour, were enriched with sulfosulfuron by growing in minimal medium. Discrete and pure colonies of SA2 were transferred to 50 ml nutrient broth and incubated at 30^0C on incubator shaker at 100 rpm. Sulfosulfuron degrading bacterial cultures were isolated using MSM (minimum salt medium) agar plates containing 50ppm sulfosulfuron as described by Xu *et al.* (2008) and Negi *et al.* (2014). The organism could utilize sulfosulfuron as a carbon and energy source. Degradation studies of sulfosulfuron were monitored by high performance liquid chromatography (HPLC - Dionex) at Department of Chemistry of the University.

Identification of sulfosulfuron degrading strain

For molecular characterization genomic DNA of strain SA2 was extracted according to Bazzirao *et al.* (1995) 16S rDNA was amplified using the Universal primers, 5'-AGAGTTTGATCCTGGCTCAG-3' and 5'-TACCTTGTTACGACTT-3'. The PCR conditions were initial denaturation at 95^0C for 1 min, annealing temperature 55-51^0C (touchdown), followed by 35 cycles of denaturation at 94^0C for 30 sec, annealing at 51^0C for 30 sec, and extension at 72^0C for 1 min, with the last cycle followed 10 min extension at 72^0C. The PCR product was run on an agrose gel and eluted using extraction kit (Genei).

After quantification, PCR product was sent to Advanced Biotechnology Centre (CIF), Delhi University South campus, India for sequencing. The resulting sequence was compared with the sequences in the GenBank database by BLAST.

Multiple sequence alignment for the homologous sequence was analyzed by MEGA 5.0 software, and a phylogenetic tree was constructed using neighbour joining method (Tamura *et al.*, 2007).

Optimization of culture condition for sulfosulfuron biodegradation

Response surface methodology (RSM) was explored to optimize the degradation conditions of sulfosulfuron using strain SA2. The Central composite design consisting of 15 experimental runs with three replicates at the center point was used to optimize the independent variables which significantly influenced the sulfosulfuron biodegradation with *Bacillus Spp*. Three critical factors and their optimal ranges were selected in this experiment-temperature (28–38^{0}C), pH of the medium (4–8) and of shaking speed 90–110 for the analysis of sulfosulfuron biodegradation (Table 1). The dependent variable was degradation of 50 ppm sulfosulfuron in 50 ml of minimal medium at 15 day.

Chemical analysis

Residual analysis of sulfosulfuron in collected samples was performed according to Anastassiades *et al.* (2003). Residual pesticide was extracted by adding either 5mL of culture broth or 5 g of soil to 20 ml acetone in a flask. Mixture was then filtered after shaking for 1 h using Buchner funnel and obtained residue was washed thoroughly with 10 ml acetone and filtered. Filtrate was collected in a round bottom flask. The residual analysis of the pesticide was done using HPLC attached with C18 reverse phase column and UV detector. A mixture of acetonitrile and water (70:30, v/v) was used as a mobile phase at a flow rate of 1.0 mL min^{-1} with the injection volume of 10 μL.

Results and Discussion

In the present study, a bacterial isolate SA2 able to utilize sulfosulfuron as a carbon source for its growth was isolated from the pesticide contaminated soil of agricultural fields of Udham Singh Nagar Uttarakhand, India. The organism was aerobic, gram positive and rod shaped. Colonies grown on nutrient agar plates were rough, opaque and dirty white. The organism degraded 80 % of 50 ppm sulfosulfuron within 15 days under shaking conditions in minimal medium. Analysis of the 16S rDNA gene sequences demonstrated that SA2 was among *Bacillus* species and was allotted with an accession number KM244748 after retrieving the sequence from NCBI database (Fig. 1 phylogenetic tree).

Optimization of sulfosulfuron degradation conditions for strain SA2

Box-Behnken design was used to determine the effect of 3 important variables (temperature (X1), pH (X2) and Shaking speed (X3)) on biodegradation of sulfosulfuron. The experimental design and the response of dependent variable for sulfosulfuron are described in the table 1. Data from table 1 was processed by response surface regression procedure of Design Expert 6.0.10 software, and results were obtained by fitting with the quadratic model equation:

$$\% \text{ Biodegradation SA2} = +75.02 + 3.71 * A + 3.06 * B + 3.53 * C + 0.75 * AB - 2.33 * AC + 1.12 * BC - 1.8 * A^2 - 6.91 * B^2 + 2.02 * C^2$$

Where (Y) is the predicted sulfosulfuron degradation (%) by strain SA2 and X1, X2, and X3 are the values for temperature, pH and Shaking speed respectively. Analysis of varience (ANOVA) for sulfosulfuron degradation is prensented in table 2. Determination coefficient $R^{2=}0.8863$ indicated that approximately 88 % of responses were covered by the model, demonstrating that predicted values of the model were in good agreement with the experimental values. The model for

sulfosulfuron biodegradation is highly significant ($p<0.0001$), indicating that the established quadratic model for sulfosulfuron degradation by SA2 was adequate and reliable in representing the actual relationship between response and variables.

Response surface methodology (RSM) is a powerful statistical technique for investigating the interactive effects between several factors at different levels and has been successfully employed to optimize removal of pollutants (Pang *et al.,* 2011). The actual responses are fitted to a polynomial model in a range of optimal responses. The model then determines the relationship between the responses and the variables and calculates the optimal responses and variables (Abdollahi *et al.,* 2012). Chandana et al (2011) reported the optimization of phenol degradation from *pseudomonas aeruginosa* (ncim 2074) using response surface methodology.

Table.1 Box-Behnken design experimental design and the response of dependent variable for pesticide degradation with bacterial strain

Run	A	B	C	% Biodegradation of Sulfosulfuron
1	-1	1	0	65
2	0	0	0	78
3	-1	0	-1	66
4	-1	-1	0	61
5	1	0	1	78
6	-1	0	1	78
7	0	0	0	78
8	0	0	0	77
9	1	1	0	76
10	1	-1	0	66
11	0	-1	-1	65
12	0	1	1	64.7
13	0	1	-1	71
14	0	0	0	76
15	1	0	-1	83
16	0	-1	1	75
17	0	0	0	80

Table.2 ANOVA for the fitted quadratic polynomial model of sulfosulfuron degradation with SA2

Source	SS	DF	MS	F-Value	p-value	
Model	994.95	9	110.55	11.53	0.0020	significant
A-Temperature	311.25	1	311.25	32.47	0.0007	
B-pH	321.31	1	321.31	33.52	0.0007	
C-Shaking speed	146.21	1	146.21	15.25	0.0059	
AB	6.25	1	6.25	0.65	0.4459	
AC	42.90	1	42.90	4.48	0.0722	
BC	44.22	1	44.22	4.61	0.0688	
A^2	0.89	1	0.89	0.093	0.7693	
B^2	113.42	1	113.42	11.83	0.0108	
C^2	6.22	1	6.22	0.65	0.4471	
Residual	67.09	7	9.58			
Lack of Fit	16.88	3	5.63	0.45	0.7321	not significant
Pure Error	50.21	4	12.55			
Cor Total	1062.04	16				

*R^2=0.9368, Std. Dev= 3.10, C.V%=4.54
DF degrees of freedom, SS sum of squares
*P level less than 0.05 indicates that the model terms are significant

Fig.1 Phylogenetic analysis using MEGA 5.0

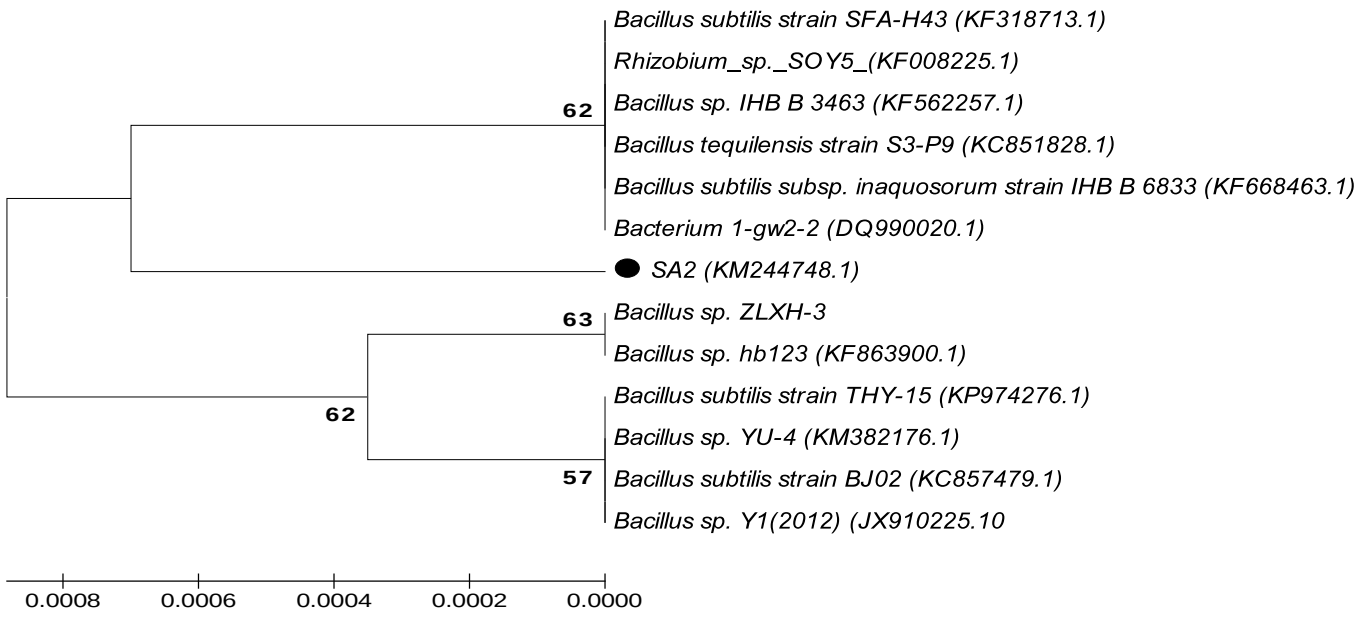
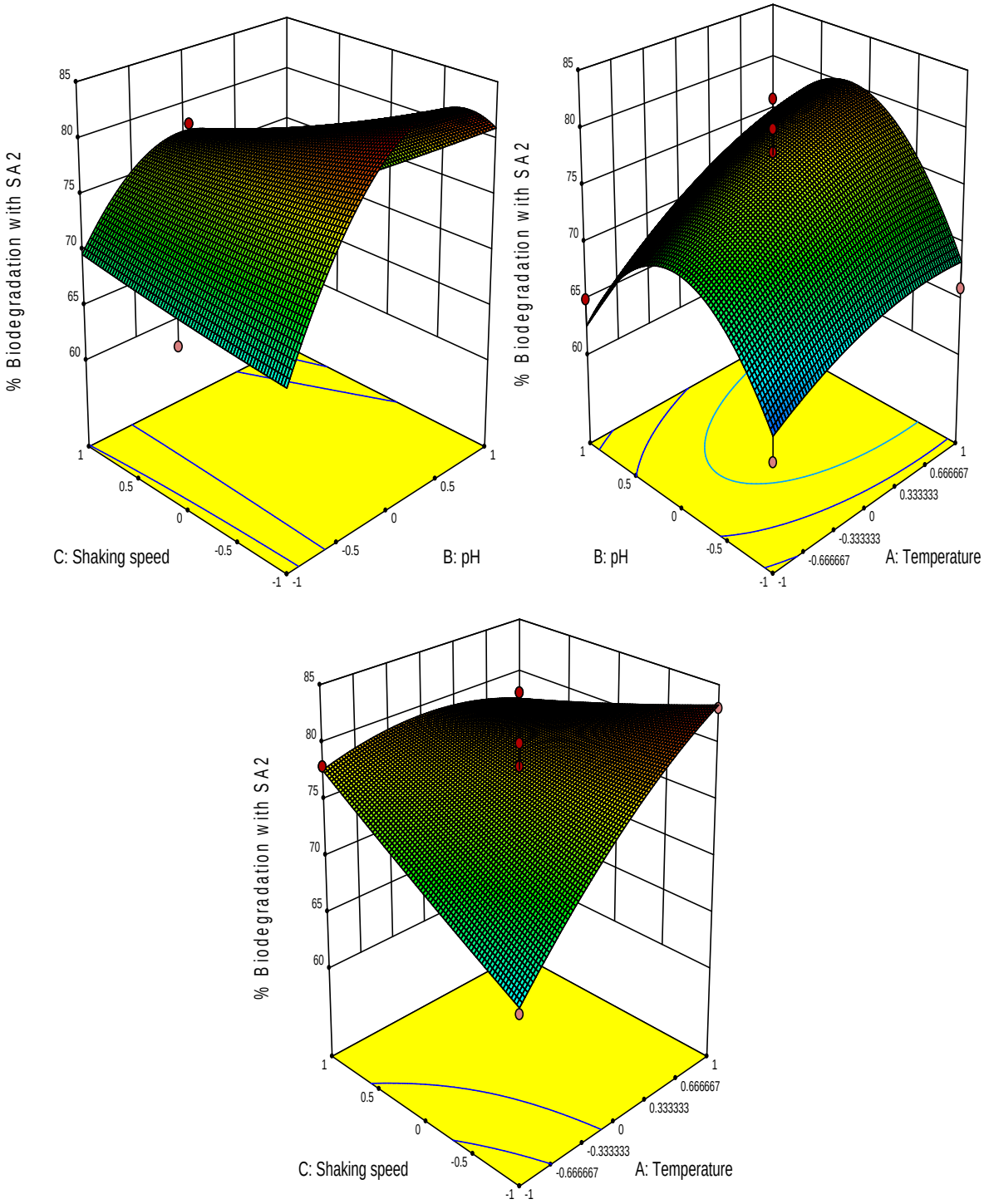


Fig.2 Optimization of sulfosulfuron biodegradation



Full factorial Central Composite Design was employed combining with Response Surface Methodology (RSM) to optimize the process parameters for the degradation of phenol by *P. aeruginosa* (NCIM 2074). They observed that a second order polynomial regression model could properly interpret the experimental data with an R^2 value of

0.9669 and an F-value of 32.52295 based on which the maximum degradation of phenol was estimated up to 80.45% within the range examined. Mansouriieh *et al.* (2015) reported the synthesized bimetallic Fe/Ni nanoparticles and used them for catalytic degradation of profenofos, an organophosphorus pesticide. A three-factor central composite design combined with response surface methodology was used to maximize profenofos removal using the bimetallic system. A quadratic model was built to predict degradation efficiency. ANOVA was used to determine the significance of the variables and interactions between them. Good correlation between the experimental and predicted values was confirmed by the high F-value (16.38), very low P-value. Schenone *et al.* (2015) reported that Modeling and optimization of photo-Fenton degradation of 2,4-D using ferrioxalate complex and response surface methodology (RSM). Biodegradation of 2,4-D w was optimized by the application of a three-level factorial experimental design combined with the Response Surface Methodology (RSM). The significance of models and their coefficients were assessed with the analysis of variance (ANOVA).

In conclusion, the strain SA2 has the ability to maximum degradation of sulfosulfuron in minimal medium after 15 days of experiment. It was observed that by response surface methodology, there was several possibility of degradation using different set of experiment. The strain might be used for the degradation of other pesticides also, and can be utilized for large scale degradation of pollutants from the environments.

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