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Co-metabolic degradation of bensulfuron-methyl in laboratory conditions

Wei Luo[a], Yuhua Zhao[a,*], Haitao Ding[a], Xiaoyan Lin[a], Huabao Zheng[a,b]

[a] Institute of Microbiology, College of Life Science, Zhejiang University, Hangzhou 310029, PR China
[b] Max-Planck-Institut für Kohlenforschung, Kaiser-Wilhelm-Platz 145470 Mülheim/Ruhr, Germany

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ABSTRACT

The present study deals with the degradation of bensulfuron-methyl by microorganisms cultured with different sources of carbon, nitrogen and phosphorus. Addition of carbon source accelerated the degradation of bensulfuron-methyl under co-metabolism process. Sodium lactate was the best carbon source for the degradation of bensulfuron-methyl, compared to other carbon sources studied, and the degradation ratio of bensulfuron-methyl reached 79.5%, whereas only 34.6 and 29.7% were removed in the presence of glucose and sucrose, respectively. Supplement of nitrogen source also enhanced degradation of bensulfuron-methyl. However, no significant differences were observed in the loss of bensulfuron-methyl between organic nitrogen and inorganic source. Phosphate buffer was supplemented into the media to maintain neutral conditions for the advantage of the strain growth since increase in pH value was observed. An orthogonal array design was applied to arrange main factors singled out for investigating the influence of factor and interaction between them on the degradation of bensulfuron-methyl. Statistical analysis showed that the concentration of sodium lactate, bensulfuron-methyl and inoculum size were the main effects, and the interaction of sodium lactate and bensulfuron-methyl was of statistical significance.

1. Introduction

Bensulfuron-methyl (BSM, 2-(4, 6-dimethoxypyrimidin-2-carbamoylsulfamoyl)-*o*-toluic acid methyl ester), discovered by the DuPont Co. in the late 1970s, is a type of sulfonylureas herbicide, characterized by broad-spectrum weed control at very low use rates (2–100 g ha^{-1}), high herbicidal activity, good crop selectivity and low mammalian toxicity [1–4]. All these characteristics make the sulfonylureas such as BSM an ideal substitute for other older herbicides in weed control [5]. The use of BSM in southeast of Asia, California and many other regions as a rice herbicide has steadily increased over the past several years [6–8], making great contribution to crop protection and production. However, the low volatility and photodegradation of BSM and the long persistence (over 100 days) in certain pedo-climatic conditions (dry climates, alkaline soils) has raised increasing concerns about the leaching or residues with risk of contamination of nearby aquatic systems or damage in the crop rotation [9].

Decay of BSM in soil mainly undergoes a process of chemical hydrolysis and biological degradation [10], while other dissipation processes such as volatilization and photolysis are relatively insignificant [9]. The hydrolysis of BSM is pH-dependent with the characteristic that the hydrolysis rate increases with a decreasing pH and stays at a minimal level around pH 8, which elucidates the phenomenon that hydrolysis of BSM is the main degradation in acidic soil conditions. At neutral or acidic conditions (pH < 8), hydrolysis of the herbicide BSM involves only the breakdown of the sulfonylurea bridge with the formation of stable pyrimidinamine and benzylsulfonamide. The latter compound is easily cyclized (pH ≥ 6) [11]. The biological degradation of herbicide in soil, mainly referring to microbial degradation, implies the use of microorganisms including, eukaryotes such as fungi, algae, yeasts and prokaryotes. Reports that the decay of sulphonylureas is more effective and faster in non-sterile compared to sterile soil reveal that the process mainly depends on the soil microflora [12,13].

In natural conditions, the constituents of soil are complex, including kinds of nutrition elements that could be utilized by soil microorganism, so the easily biodegradative substances may be preferred as sources of carbon and energy to the toxic herbicide, resulting in co-metabolic degradation of herbicide. Co-metabolism describes the ability of microorganisms to remove non-growth supporting substrates, typically in the presence of a growth supporting substrate [14–17], which is of vital importance in the disappearance of the pollutant from the contaminated environment. Bioremediation of contaminated environment, especially the microbial bioremediation in virtue of co-metabolism has received

* Corresponding author. Tel.: +86 571 8820 8557.
E-mail address: yhzhao_1958@yahoo.com.cn (Y. Zhao).

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increasing attention as an effective biotechnological approach to clean up pollution because of its effectiveness and low-cost.

In this work, we investigated the principles of co-metabolic degradation of BSM by *Brevibacterium* sp. strain under different sources of carbon, nitrogen and phosphorus.

2. Materials and methods

2.1. Chemicals

BSM (analytical grade ≥98.5%) was purchased from DuPont Co. (USA); HPLC-grade acetonitrile was from SiYou Inc. (Tianjin, China). Other chemicals (Tables 1 and 2) used were of analytical grade.

2.2. Strain, media and culture conditions

The strains used in this study belong to genus *Brevibacterium*. Light microscope observations showed that they were non-motile short rods in shape. Cells were generally 0.6–1 μm in diameter. Colonies of the strain, formed on LB plate were round, smooth, sticky, white-yellow in color. The optimal temperature and pH for the growth were 30 °C and 7.2, respectively.

The growth mineral medium for the *Brevibacterium* sp. strain was a modification of the M9 medium [18], containing ($g\,l^{-1}$) $Na_2HPO_4{\cdot}12H_2O$, 17.1; KH_2PO_4, 3.0; NH_4Cl, 1.0; NaCl, 0.5; $MgSO_4{\cdot}7H_2O$, 0.492; $CaCl_2{\cdot}2H_2O$, 0.026; $FeCl_2{\cdot}2H_2O$, 0.08. All samples were placed in 500 ml Erlenmeyer flasks containing 100 ml medium buffered at pH 7.2 incubated with shaking (150 rpm) at 30 °C. Considering that the pH value increased with incubation time, phosphate buffer at different concentration was investigated. Different concentrations of phosphate buffer (0.05, 0.1, 0.2 mol l^{-1} Na_2HPO_4–KH_2PO_4 and 0.01, 0.02, 0.05 mol l^{-1} KH_2PO_4) were studied.

2.3. Inoculum preparation

The strain was incubated in 500 ml Erlenmeyer flaks containing 100 ml LB medium (peptone, 10 g l^{-1}; yeast extract, 5 g l^{-1}; NaCl, 10 g l^{-1}; pH 7.2) supplemented with 20 mg l^{-1} BSM. Strains were collected at exponential phase by centrifugation at 12,000 rpm for 2 min, then the precipitate was washed twice with sterilized physiological saline (0.9% NaCl), before the preparation of cell suspension by the same solution.

2.4. Extraction of BSM and high-performance liquid chromatography (HPLC) assay

A 1.5 ml aliquot of the culture was transferred from each liquid medium at the same interval, stored at −20 °C for further assay. Samples were extracted with CH_2Cl_2 for three times (3× 2 ml), then the organic phases were collected in test tubes, and concentrated to dryness with pure N_2 under low temperature before dissolving it in acetonitrile.

Samples were analyzed by HPLC after filtration of the acetonitrile solution through nylon filters (0.22 μm, Tengda Inc., Tianjin, China). HPLC analysis of BSM were carried out using an Agilent 1100 HPLC instrument (Agilent Technologies Co. Ltd., USA), equipped with an UV variable wavelength detector set at 238 nm. HPLC separation was performed on a C_{18} reversed phase column (TOSOH, Japan), 150 mm × 4.6 mm, 5 μm particle size. The operating conditions were mobile phase acetonitrile/water (55:45, v/v) containing 0.1% phosphoric acid, isocratic; flow rate 0.8 ml min^{-1}; column temperature 35 °C; volume injected 20 μl. Variation coefficients were lower than 5% in all case. Triplicate analyses were conducted and the recoveries of BSM from liquid media at levels of 25, 50 and 100 mg l^{-1} were determined to be 93.1 ± 4.1, 98.2 ± 2.5 and 96.4 ± 3.7%, respectively. These data indicated that the extraction procedure was efficient in extracting BSM from liquid media.

2.5. Experiment procedures and statistical analyses

Firstly, the "one-factor-at-a-time" approach was applied to investigate the effect of different sources of carbon, nitrogen and phosphorus on the degradation of BSM. The used amounts are shown in Tables 1 and 2. Biodegradability tests were carried out independently in triplicate and the average values were calculated. Pseudo-first-order rate constants for the reactions were obtained using the equation as follows:

$$\ln\ c_t = -kt + \ln\ c_0$$

where c_0 is the substrate concentration at zero time and c_t is the substrate concentration at time t. The rate constant was k.

Orthogonal array design (OAD), known as Taguchi design, is believed to incorporate the advantages of simplex method and factorial design. It is very efficient in reducing the number of experiment, gaining the desired trial result and generating useful information on key variable [19,20]. In the present work, a L_{27} (3^{13}) OAD procedure was performed with four factors at three levels to study the complex influence of process parameters on the degradation efficiency of BSM by *Brevibacterium* sp. strain.

A SAS software package was used to do the analysis of variance, so as to obtain the main effect and interactions between process parameters. Data obtained were also subjected to observation analysis in order to determine the influence of parameters on output response.

3. Results and discussions

3.1. Co-metabolic characteristics of BSM in the presence of different carbon sources

The degradation of BSM supplied with different carbon sources (Table 1) was studied. As shown in Fig. 1a, during the first several days, there was nearly no degradation of BSM and the stationary phase (lag-phase-like-period) was longer in the media that used BSM as the sole carbon source than those supplemented with other carbon sources. It is suggested that high concentration of BSM would cause toxicity to the growth of soil bacteria. Gigliotti et al. [21] evidenced the addition of BSM at a 10-fold field rate would significantly decrease the number of cellulose-degrading bacteria. A negative effect of BSM on soil microbial activity, may be attributed to the toxicity of herbicide to the indigenous microorganisms [22,23], which was also found in other sulfonylurea herbicides [23]. Supplement of other carbon sources might relieve the toxicity effect of BSM on *Brevibacterium* sp. by supplying nutrition and shortening the adaptation time.

The data shown in Fig. 1a were fitted using first-order reaction kinetic model and the results revealed that biodegradation of BSM in the presence of different carbon sources agreed well with first-order equation (Table 1). The kinetic results showed that the media supplemented with different carbon sources presented a significantly higher BSM degradation rate than the ones that used BSM as a single carbon source. The half-life of BSM in the presence of sodium lactate was only 11.4 days, compared to those with or without other carbon sources. Degradation fraction of BSM was from 29.7 to 79.5% (Table 1), with the maximum in the presence of sodium lactate, which suggested that BSM degradation underwent a co-metabolic process, since the growth substrate enhanced the metabolism of co-substrate.

Table 1
Kinetic results of BSM in the presence of different carbon sources

Carbon source	Added amount ($g\,l^{-1}$)	Kinetic equations	Degradation rate constant (k, day^{-1})	Correlation coefficient r^2	Half-life of BSM (days)	Degradation fraction of BSM (%)
Sodium formate	2.0	$\ln c = -0.0289t + 4.6297$	0.029	0.978	24.0	53.5
Sodium acetate	2.0	$\ln c = -0.0431t + 4.6745$	0.043	0.961	16.1	67.7
Sodium lactate	2.0	$\ln c = -0.061t + 4.7316$	0.061	0.972	11.4	79.5
Sodium citrate	2.0	$\ln c = -0.0468t + 4.6297$	0.047	0.946	14.8	70.7
Glucose	2.0	$\ln c = -0.0153t + 4.5104$	0.015	0.955	45.3	34.6
Sucrose	2.0	$\ln c = -0.0119t + 4.539$	0.012	0.982	58.2	29.7
Control 1[a]	–	$\ln c = -0.008t + 4.606$	0.008	0.943	86.6	18.6
Control 2[b]	–	$\ln c = -0.0046t + 4.5166$	0.005	0.992	150.7	12.8

[a] BSM as the sole carbon source with strains.
[b] BSM as the sole carbon source without strains.

Table 2
Kinetic results of BSM in the presence of different nitrogen sources

Nitrogen source	Added amount ($g\,l^{-1}$)	Kinetic equations	Degradation rate constant (k, day^{-1})	Correlation coefficient r^2	Half-life of BSM (days)	Degradation fraction of BSM (%)
NH_4Cl	1.0	$\ln c = -0.0505t + 4.7238$	0.051	0.968	13.7	73.9
$NaNO_3$	1.0	$\ln c = -0.0405t + 4.6851$	0.041	0.962	17.1	64.1
Yeast extraction	1.0	$\ln c = -0.0418t + 4.6585$	0.042	0.982	16.6	67.1
Peptone	1.0	$\ln c = -0.0566t + 4.7329$	0.057	0.983	12.2	77.4

Co-metabolism, as a very universal phenomenon in nature, has been studied extensively in the degradation of pollutants. Meng et al. [24] showed that the loss of omethoate was enhanced effectively by the way of co-metabolism. Hess et al. [25] also reported that the degradation rate of 2,4-dinitrophenol was enhanced by the presence of glucose. The mechanisms of co-metabolic degradation of pollutants by microbe involve the following processes [26]: first, utilization of growth substrate by microbe and production of broad substrate specificity of catabolic enzymes (induced enzymes); second, imperfect coupling between enzyme specificity and gene regulation; third, metabolic interdependence of growth substrate and co-substrate; and fourth, growth substrate effects on the metabolism of co-substrate. In our experiments, sodium lactate was the best growth substrate, and resulted in a much better degradation fraction than that of glucose and sucrose. The result was much similar to the study described by Meng et al. [24]. It was possible that BSM hydrolase acted as an induced enzyme and its expression was inhibited by reducing sugar such as glucose and sucrose or their metabolic products.

Since degradation of BSM was pH-sensitive, pH value was detected during the process of culture. With the metabolism of BSM and/or other carbon sources, the value of pH increased from 7.2 to more than 9.0 (data not shown). The results suggested that the strain was adapted to pH change and the effect of pH variation on the chemical hydrolysis of BSM was insignificant in this work.

3.2. Effect of nitrogen source on BSM co-metabolic degradation

In our experiments, the influence of inorganic and organic nitrogen sources on the degradation of BSM was studied in the presence of sodium lactate. As shown in Fig. 1b, it was obvious that the lag phase was shorter in the media with organic source, than that of the ones in the presence of inorganic source. This might be attributed to the excess supply of other nutrition elements in addi-

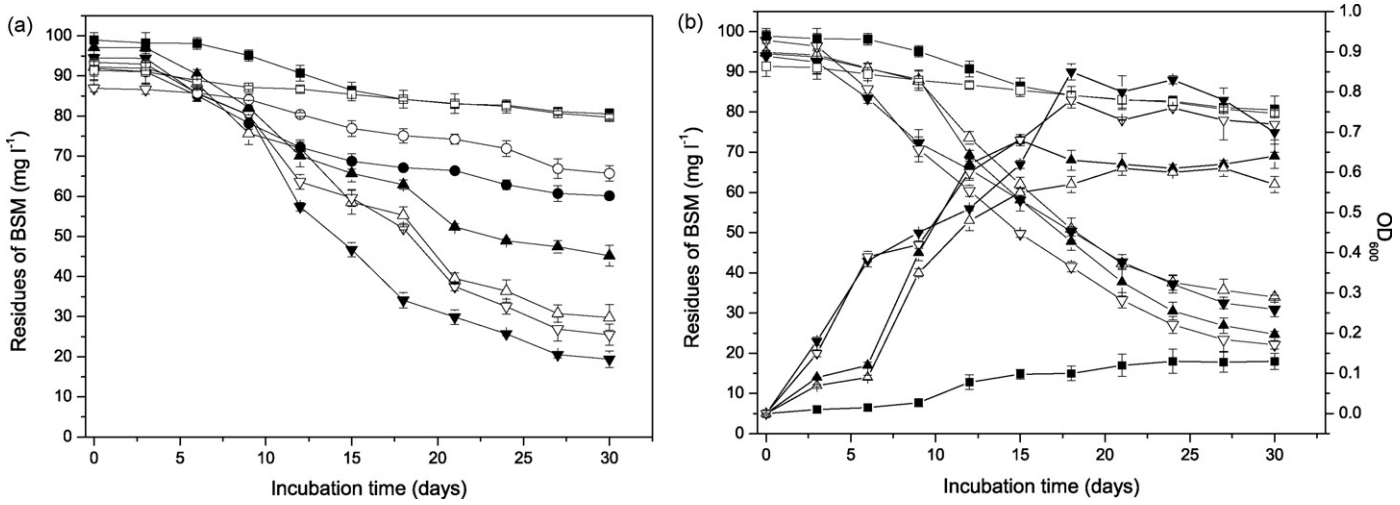

Fig. 1. (a) Degradation of BSM in the presence of different carbon sources, (▲) sodium formate, (△) sodium acetate, (▼) sodium lactate, (▽) sodium citrate, (●) glucose, (○) sucrose, (■) BSM as the sole carbon source with strains, (□) BSM as the sole carbon source without strains; (b) degradation of BSM within different nitrogen sources, (▲) NH_4Cl, (△) $NaNO_3$, (▼) yeast extraction, (▽) peptone, (■) BSM as the sole nitrogen source with strains, (□) BSM as the sole nitrogen source without strains and growth amounts of the strains within aforementioned nitrogen sources.

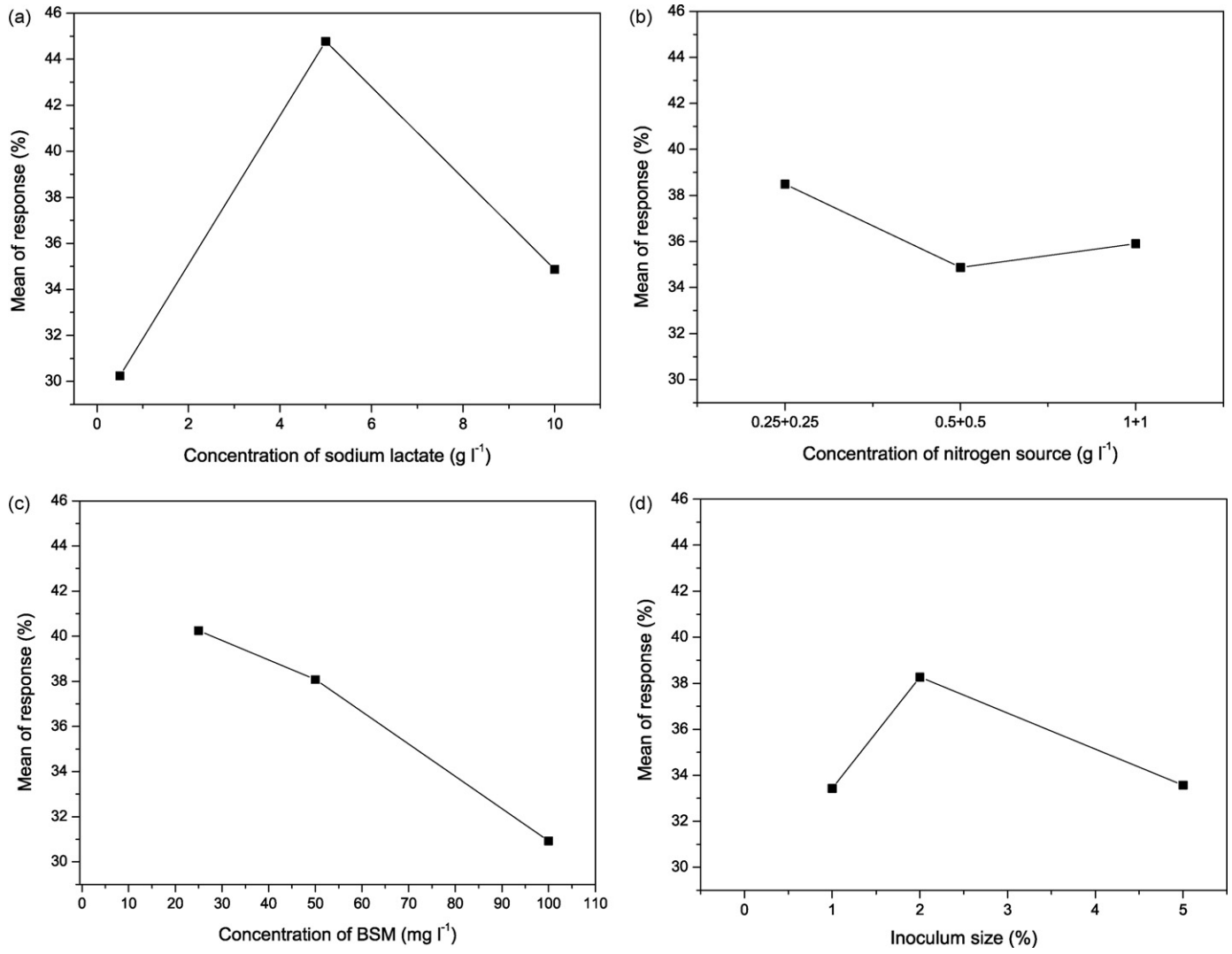

Fig. 2. Effects of (a) sodium lactate, (b) nitrogen source, (c) BSM and (d) inoculum size on the degradation of BSM.

tion to N element in media. Compared the media with inorganic nitrogen source, ones supplemented with organic nutrition would promote the growth of strains, relieve toxicity of the herbicide and curtail adaptation time. The degradation rate of BSM increased dramatically for the first 10 days, while it decreased rapidly, which was not the same with those of the media added of inorganic nitrogen sources. Supplement of nutrition substance significantly accelerated the growth of microorganisms (see Fig. 1b), increased the biomass of bacteria and promoted the expression of induced enzyme that was able to co-metabolically remove the herbicide. Meanwhile, the affinity of the induced enzyme to first substrate was often much higher than that to the co-substrate [27], so the superabundant growth substrate would give rise to inhibition between substrates, with the result of decrease in degradation rate of BSM. The data shown in Fig. 1b were well fitted using first-order reaction kinetics model and the results were summarized in Table 2. The degradation rate constants of BSM in the presence of NH_4Cl and peptone were higher than those within other nitrogen sources, which resulted in a shorter half-life of BSM in the former media. The degradation fractions of BSM were finally determined 73.9 and 77.4%, respectively. In our study, addition of NH_4Cl and peptone obtained better degradation of BSM, so they were singled out for further study.

3.3. Influence of phosphate on the metabolism of BSM and pH value

Buffer of 0.2 mol l^{-1} Na_2HPO_4–KH_2PO_4 presented stronger cushion ability than others in the initial phase of incubation time, however, after 20 days incubation the pH value also increased rapidly to the ultimately same level as others, which meant substance with strong alkalescence coming into being. However, the degradation fraction of BSM in different media presented no significant difference (data not shown).

3.4. Result analysis of orthogonal array design

3.4.1. ANOVA analysis of the OAD

In the present study, four process parameters, i.e., sodium lactate, nitrogen source (NH_4Cl + peptone), BSM and inoculation size were considered. The process parameters and their selected levels were randomly distributed as presented in Table 3. Considering the degradation efficiency and tolerance of the strains to BSM, 25 and 100 mg l^{-1} of BSM were set as the lower limit and upper limit, respectively.

For four parameters at three levels each, the full factorial design would require 3^4 (81) experiments. However, consider-

Table 3
Selected process parameters and their respective levels

Process parameters	Symbol	Level 1	Level 2	Level 3
Nitrogen source (NH_4Cl + peptone) ($g\,l^{-1}$)	*A*	0.5 + 0.5	0.25 + 0.25	1 + 1
Sodium lactate ($g\,l^{-1}$)	*B*	10	5	0.5
BSM ($mg\,l^{-1}$)	*C*	100	50	25
Inoculum size (%)	*D*	1	5	2

Table 4
Assignment of factors and levels by using a L_{27} (3^{13}) matrix along with the response

Trial number	*A*	*B*	$(C \times D)_2$	*e*	*C*	*e*	*e*	$(B \times C)_1$	*D*	*e*	$(B \times C)_2$	*e*	$(C \times D)_1$	Response (%)
	1	2	3	4	5	6	7	8	9	10	11	12	13	
1	1	1	1	1	1	1	1	1	1	1	1	1	1	20.79
2	1	1	1	1	2	2	2	2	2	2	2	2	2	36.03
3	1	1	1	1	3	3	3	3	3	3	3	3	3	35.56
4	1	2	2	2	1	1	1	2	2	2	3	3	3	28.07
5	1	2	2	2	2	2	2	3	3	3	1	1	1	48.38
6	1	2	2	2	3	3	3	1	1	1	1	1	1	52.11
7	1	3	3	3	1	1	1	3	3	3	2	2	2	35.27
8	1	3	3	3	2	2	2	1	1	1	3	3	3	28.78
9	1	3	3	3	3	3	3	2	2	2	1	1	1	28.83
10	2	1	2	3	1	2	3	1	2	3	1	2	3	36.43
11	2	1	2	3	2	3	1	2	3	1	2	3	1	35.39
12	2	1	2	3	3	1	2	3	1	2	3	1	2	40.39
13	2	2	3	1	1	2	3	2	3	1	3	1	2	34.58
14	2	2	3	1	2	3	1	3	1	2	1	2	3	53.63
15	2	2	3	1	3	1	2	1	2	3	2	3	1	57.10
16	2	3	1	2	1	2	3	3	1	2	2	3	1	28.38
17	2	3	1	2	2	3	1	1	2	3	3	1	2	30.80
18	2	3	1	2	3	1	2	2	3	1	1	2	3	29.74
19	3	1	3	2	1	3	2	1	3	2	1	3	2	35.04
20	3	1	3	2	2	1	3	2	1	3	2	1	3	30.40
21	3	1	3	2	3	2	1	3	2	1	3	2	1	38.20
22	3	2	1	3	1	3	2	2	1	3	3	2	1	24.12
23	3	2	1	3	2	1	3	3	2	1	1	3	2	46.99
24	3	2	1	3	3	2	1	1	3	2	2	1	3	58.07
25	3	3	2	1	1	3	2	3	2	1	2	1	3	35.72
26	3	3	2	1	2	1	3	1	3	2	3	2	1	32.37
27	3	3	2	1	3	2	1	2	1	3	1	3	2	22.26

ing the interactions between process parameters, only 27 (L_{27} orthogonal array) experiments were required in the present design (Table 4).

As shown in Table 5, ANOVA of OAD was obtained using SAS package and the results for whose *P* was lower or close to 0.05 were shown. The ANOVA results indicated that sodium lactate, BSM and the interaction between them were statistically significant at $P < 0.0001$. The inoculum size was also statistically significant at $P < 0.01$. In the meantime, no statistical differences were observed for nitrogen source and the interaction between BSM and inoculum size at $P > 0.05$. These results were further confirmed by the analysis of percentage contribution (PC) contained in Table 5. The results indicated that sodium lactate was the most important factor contributing to the out response (37.08%) and then followed by interaction between sodium lactate and BSM (32.14%), BSM (15.29%), inoculum size (3.99%). The percent contributions of the nitrogen source and the interactions between BSM and inoculum size were quite low, thus they could be pooled as error. Obviously, the data analysis of PC% was in good agreement with the conclusion from the significance analysis.

Table 5
Results of ANOVA including percentage contribution for output response in the L_{27} (3^{13}) matrix

Source	ss[a]	d.f.[b]	Ms[c]	*F*[d]	*P*	SS′[e]	PC (%)[f]
A	62.713	2	31.357	4.00	0.0529	47.039	1.74
B	1015.663	2	507.831	64.80	<.0001	999.989	37.08
C	427.961	2	213.981	27.30	<.0001	412.287	15.29
D	123.184	2	61.592	7.86	0.0089	107.51	3.99
$B \times C$	897.900	4	224.475	28.64	<.0001	866.552	32.14
$C \times D$	90.774	4	22.693	2.90	0.0787	59.426	2.20
Error	78.374	10	7.837	–	–	203.966	7.56
Total	2696.569	26	–	–	–	2696.569	100

[a] Sum of square.
[b] Degrees of freedom.
[c] Mean square.
[d] *F* value.
[e] Purified sum of square.
[f] Percentage contribution.

Table 6
The sum (K) and average (k) at each level of a factor and range of response

	Sodium lactate	Nitrogen source	BSM	Inoculum size
K_1	308.232	313.821	278.397	300.861
K_2	403.038	346.437	342.756	338.157
K_3	272.151	323.163	362.259	344.403
k_1	34.248	34.869	30.933	33.429
k_2	44.782	38.493	38.084	33.573
k_3	30.239	35.907	40.251	38.267
Range	14.543	3.624	9.318	4.838

3.4.2. Direct observation analysis of the OAD

Results of the OAD experiment could also be statistically treated in direct observation analysis to assess the influence of different process parameters. The sum (K) and average (k) of response at each level of every factor was calculated from the data listed in Table 4 and the results are listed in Table 6.

Fig. 2a shows that the effect of sodium lactate on the degradation of BSM was very considerable. The range of mean response at each level was up to 14.5%, dramatically influencing the degradation of BSM. The degradation efficiency increased in company with the rising concentration of sodium lactate from 0.5 to 5 g l^{-1}, but decreased rapidly whereas the latter increased. This was attributed to the interaction between sodium lactate and BSM. The ANOVA results also show the interaction of the two parameters was statistically significant. Interaction plot between sodium lactate and BSM was shown in Fig. 3. Through the chart, it was possible to realize that the effect of sodium lactate on the response was different at different levels of BSM. At 25 and 50 mg l^{-1} of BSM, effects of sodium lactate on BSM degradation were obvious and the trend was consistent with the one in Fig. 2a. The maximum mean response was achieved when sodium lactate was 5 g l^{-1} at 25 mg l^{-1} of BSM, followed by at 50 mg l^{-1}. In the both conditions, degradation of BSM exhibited a hill shape. The phenomenon could be elucidated by competitive inhibition, accompanied by induced enzyme action on the substrates. The mechanism of co-metabolism lies in the non-specific enzyme, induced by the growth substrate, simultaneously transforming co-substrate [16,26,28]. In the degradation process, growth substrate and co-substrate could competitively binds to the finite active site of induced enzyme and result in competitive inhibition between substrates. The competitive inhibition effect between substrates is slight when the concentration ratio at an appropriate level. In our study, growth substrate at relative low level could promote the degradation of co-substrate. As shown in Fig. 2a, with the increase of sodium lactate from 0.5 to 5 g l^{-1} in the media, the co-substrate BSM presented a rising degradation. This was in accordance with other literatures [29]. However, when the concentration of growth substrate is much higher than co-substrate, competitive inhibition presents obvious. Anke and Edward [27] reported that degradation of *cis*-1,2-dichloroethylene and vinyl chloride was obviously inhibited initially by growth substrate toluene at high levels. Other similar conclusions were also reported [14,30,31]. Therefore, the provision of a growth substrate in a co-metabolism system became a tradeoff between undesirable effect of competitive inhibition and cell growth to yield more induced enzyme to over the effect. A suitable concentration ratio between growth substrate and co-substrate should be selected for the maximal degradation of pollutants.

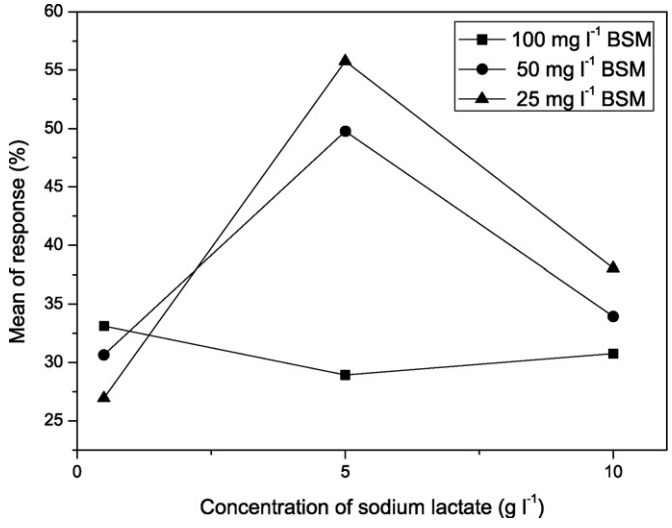

Fig. 3. Effects of the interaction between sodium lactate and BSM on the response (mean value).

Fig. 2c indicates the relation between the degradation fraction and concentration of BSM. The mean response decreased with the increasing concentration of BSM. The best level was 25 mg l^{-1} of BSM, with the best degradation of 40.25%, whereas there was a weak decline in BSM degradation when BSM increased from 25 to 50 mg l^{-1}. However, degradation of BSM dramatically decreased accompanied with the BSM from 50 to 100 mg l^{-1}, which was much similar with the condition in Fig. 3 when sodium lactate was at 5 g l^{-1} level. With the variation of BSM in concentration, the concentration ratio of sodium lactate to BSM varied and might bring about inhibition effect; on the other hand, high concentration of BSM was toxic to strain and led to decline in metabolism ability of strains (Figs. 2c and 3). Meanwhile, at 0.5 and 10 g l^{-1} of sodium lactate (see Fig. 3), effects of BSM concentration on the degradation of BSM were not obvious. So a proper concentration should be selected in order to get a high degradation of BSM in a co-metabolic system. In our study, for a better degradation of BSM, 50 mg l^{-1} BSM was a good level, considering the degradation efficiency.

The ANOVA results show that inoculum size had statistically significant effect at $P < 0.01$. It can be seen from Fig. 2d that with the rising inoculum size from 1 to 2%, degradation of BSM increased. However, too high biomass of microorganisms resulted in decrease in degradation fraction. Although the influence of nitrogen source on the degradation of BSM was insignificant due to a limited span of nitrogen source (0.5–2 g l^{-1}) (Fig. 2b) in the present work, it could not conclude that nitrogen source did not have crucial effect on the degradation of BSM in every circumstance. Moreover, nitrogen source was necessary for the growth of microorganisms and production of induced enzyme.

4. Conclusions

In the present work, influence of different sources of carbon, nitrogen and phosphorus on the degradation of BSM was investigated and the results suggested its degradation underwent a co-metabolism way. OAD was applied to study the effects of factors on BSM degradation. The main effects and interactions between factors were analyzed, by using statistical analysis. From the results, co-metabolic degradation of BSM was in relative with concentration of sodium lactate and BSM because of the competitive inhibition existing between them. A suitable ratio of sodium lactate and BSM should be determined in order to get maximum degradation efficiency. This work has a certain practical value in the bioremediation of BSM-contaminated water and soil.

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