Optimization of fed-batch fermentation and direct spray drying in the preparation of microbial inoculant of acetochlor-degrading strain *Sphingomonas* sp. DC-6

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Abstract

Microbial inoculant preparation is a prerequisite for its application in large-scale bioremediation. In the present study, *Sphingomonas* sp. DC-6, an efficient acetochlor-degrading strain, was used to investigate the process of preparing the inoculant. Optimization of submerged fermentation (SmF) by response surface methodology (RSM) resulted in a first 22% increase in biomass of liquid inoculant. Then, a biomass increase of 2.18 times with 14.58% shortened incubation period was further obtained in optimized medium using a 7.5-l bioreactor. However, less than 0.4% viable cells in liquid inoculant survived after 180-days storage. Thus, optimized spray drying conditions were subsequently employed for the production of high viability powder (2.11×10^{12} cfu g⁻¹ powder) without additive and its survival ratio (SR) after 180-days storage was still maintained at 90.5%. Both the 180-days stored powder and the original powder showed the same degradation performance, being able to completely degrade 200 mg l⁻¹ acetochlor within 48 h. This study demonstrated that strain DC-6 was suitable for industrial production of bacteria powder and provided a potential approach for the preparation of pesticide-degrading microbial inoculant.

Keywords Acetochlor herbicides · Microbial inoculant · *Sphingomonas* sp. DC-6 · Fed-batch fermentation · Response surface methodology · Spray drying

Introduction

Acetochlor, a highly efficient preemergent herbicide for controlling the growth of common annual grasses and broadleaf weeds, is the most commonly used chloroacetanilide herbicide (Cahoon et al. 2001). Due to its widespread use, long persistence, and high water solubility, acetochlor residues are frequently detected in soil and underground water, injuring many crops (Hildebrandt et al. 2008). Additionally, acetochlor is harmful to the animals' sciatic nerve (Zafeiridou et al. 2006). Therefore, the degradation of acetochlor in the environment has received considerable attention.

Microbial degradation plays a very important role in the removal of the environmental acetochlor (Beestman and Deming 1974). Many microorganisms capable of completely or partially degrading chloroacetanilide herbicides were isolated (Saxena et al. 1987; Jia et al. 2006; Xu et al. 2006; Wang et al. 2008; Dwivedi et al. 2010; Zhang et al. 2011). Strain DC-6, one of the most excellent acetochlor-degrading strains, is able to completely degrade three chloroacetanilide herbicides including alachlor, acetochlor and butachlor (Chen et al. 2014). In addition, its degradation pathway is known and the involved genes including *cndA*, *cmeH*, *meaX*, *meaY*, *meaA* and *meaB* have been cloned (Li et al. 2013; Chen et al. 2014; Hou et al. 2014; Cheng et al. 2017).

The application of strain DC-6 in the bioremediation of acetochlor contaminated soil was also examined by Li in a laboratory-scale investigation (2015). His results showed that strain DC-6 can degrade the acetochlor in the soil in a short period of time without adverse effects on microbial communities, suggesting its potential as a large scale bioremediation of acetochlor contaminated soil. However, a good

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strain DC-6 microbial inoculant with a stable degradation ability, high product yield, long shelf life and convenient transportation is a prerequisite for its application as a large scale bioremediation.

In this study, an optimization strategy was studied for submerged fermentation (SmF) before its fed-batch fermentation in a 7.5-l bioreactor to increase the biomass of liquid inoculant. Next, best spray drying conditions were established to make an excellent bacteria powder without addition of any protective agent or carrier for convenient transportation and long shelf life. Therefore, this study provides a useful method to prepare pesticide degrading microbial inoculant by SmF and direct spray drying technique.

Materials and methods

Microorganisms and medium

Strain DC-6, originally isolated in our laboratory, was streaked out on a selective LB agar plate (containing 500 mg l⁻¹ acetochlor) and cultured at 30 °C for 5 days. To prepare the seed culture, a single colony was inoculated into the seed medium (LB broth) and left in a shaker at 30 °C, 180 r min⁻¹ for 48 h.

Liquid inoculant preparation through submerged fermentation

The fermentation variables were first selected in accordance with the single-factor experiments, and then the response surface methodology (RSM) was used for optimization through Plackett–Burman (PB) design and Box–Behnken design (BBD).

Based on the results of single-factor experiments, the following variables such as temperature, pH, inoculum amount, medium volume, speed, glucose, yeast extract/(NH₄)₂SO₄, NaCl, MgSO₄ and K₂HPO₄ were identified as ten important variables for biomass (data not shown). A PB design was initially used to analyze the effects of these variables on fermentation. Each parameter was variable at two levels, as shown in Table 1. The experiment was designed by Design Expert 8.0.6. The significance of each variable was determined using the *P*-value with biomass as the response, as shown in Table 2. Biomass measurement was described by Xu et al. (2015) with some modifications. Biomass was determined for 10 ml cell suspensions harvested by centrifugation, washed with distilled water, and dried at 60 °C for 24 h to a constant weight. This test was repeated in triplicate.

Based on the results of PB design, BBD was subsequently used to determine the optimum level of three significant factors with five replicates of the center points. Seventeen experimental runs were tested to measure the difference in

Table 1 Experimental variables at different levels used for biomass optimization of strain DC-6 using the Plackett–Burman design

Variables	Symbol code	Experimental values	
		Low (– 1)	High (+ 1)
T (°C)	<i>A</i>	28	35
pH	<i>B</i>	6.5	7.5
Inoculum amount (v/v, %)	<i>C</i>	6	9
Medium volume (ml/250 ml)	<i>D</i>	20	40
Rotation speed (r min ⁻¹)	<i>E</i>	150	200
NaCl (g l ⁻¹)	<i>F</i>	0.5	1.5
K ₂ HPO ₄ (g l ⁻¹)	<i>G</i>	0.2	0.8
MgSO ₄ (g l ⁻¹)	<i>H</i>	0.2	0.8
Glucose (g l ⁻¹)	<i>J</i>	10	20
Yeast extract/(NH ₄) ₂ SO ₄ (mol/mol, 3/1) (g l ⁻¹)	<i>K</i>	20	30

biomass (Table 3). The regression analysis and second-order polynomial coefficients were calculated using Design Expert 8.0.6 software.

The scaling up fermentation was assayed in a 7.5-l stirred bioreactor (BioFlo/CelliGen 115, New Brunswick Scientific) with a 5-l working volume. During fermentation, the inoculum amount was 7% (v/v), the aeration rate was controlled at 1.0 vvm with a constant agitation speed and all cultivations were carried out at 30 °C. The glucose content was determined by spectrophotometer at 550 nm absorbance as described by Chang and Tang (2014) and biomass was determined as described above. Each test was repeated in triplicate.

Bacteria powder preparation by spray drying

The liquid inoculant was concentrated 5 times by centrifugation, washed, and resuspended in sterile water. The best spray drying conditions were studied by single-factor and orthogonal array design methods by spray dryer (ADL311, yamato, Japan). Three operating parameters, viz. feed speed (5, 10, 15, 20 ml min⁻¹), inlet air temperature (120, 140, 160, 180, 200 °C) and hot air flow rate (0.2, 0.4, 0.6, 0.8, 1.0 m³ min⁻¹) were estimated. Optimization was operated by the L9 (3⁴) orthogonal array design.

Performance tests of liquid inoculant and bacteria powder

The ultrastructure of the inoculant was imaged using a scanning electron microscope (SU8000, Japan).

Both liquid inoculant and bacteria powder were stored at room temperature. Viable cell number was estimated by dilution plate counting method as described by French and Ward (1995). Briefly, 0.1 ml liquid inoculant or 0.1 g

Table 2 Twelve-trial Plackett–Burman design matrix for ten variables with coded values along with the tested biomass of strain DC-6

Run	A	B	C	D	E	F	G	H	J	K	Biomass (g l ⁻¹)
1	+1	-1	+1	+1	-1	+1	+1	+1	-1	-1	2.65
2	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	1.08
3	+1	-1	-1	-1	+1	-1	+1	+1	-1	+1	2.31
4	-1	+1	+1	-1	+1	+1	-1	+1	+1	+1	0.62
5	+1	+1	+1	-1	-1	-1	+1	-1	+1	+1	1.96
6	-1	+1	-1	+1	+1	-1	+1	+1	+1	-1	2.75
7	+1	+1	-1	+1	+1	+1	-1	-1	-1	+1	1.66
8	-1	-1	-1	+1	-1	+1	+1	+1	+1	+1	1.60
9	+1	+1	-1	-1	-1	+1	-1	+1	+1	-1	1.74
10	+1	-1	+1	+1	+1	-1	-1	-1	+1	-1	2.00
11	-1	+1	+1	+1	-1	-1	-1	+1	-1	+1	1.82
12	-1	+1	+1	-1	+1	+1	+1	-1	-1	-1	2.33

Table 3 BBD matrix for the experimental design with observed and predicted responses of strain DC-6 biomass

Run order	Symbol code			Biomass (g l ⁻¹)	
	X ₁ ^a	X ₂ ^b	X ₃ ^c	Observed	Predicted
1	-1	1	0	2.17	2.18
2	0	0	0	2.87	2.86
3	0	0	0	2.86	2.86
4	0	0	0	2.88	2.86
5	0	0	0	2.88	2.86
6	-1	-1	0	2.18	2.17
7	1	0	-1	2.23	2.23
8	0	1	1	2.53	2.53
9	1	0	1	2.24	2.24
10	1	1	0	2.19	2.19
11	-1	0	1	2.20	2.20
12	0	-1	1	2.51	2.51
13	1	-1	0	2.17	2.17
14	0	1	-1	2.65	2.65
15	0	-1	-1	2.64	2.64
16	0	0	0	2.86	2.86
17	-1	0	-1	2.24	2.24

^aX₁: Yeast extract/(NH₄)₂SO₄ (mol/mol, 3/1) (g l⁻¹), its coded value of -1, 0 and 1 corresponded to 20, 25 and 30, respectively

^bX₂: pH, its coded value of -1, 0 and 1 corresponded to 6.5, 7.0 and 7.5, respectively

^cX₃: K₂HPO₄ (g l⁻¹), its coded value of -1, 0 and 1 corresponded to 0.2, 0.5 and 0.8, respectively

powder was sampled and diluted in sterile water. Then, a 100 µl aliquot of the diluted cells was applied to LB agar plates supplemented with 500 mg l⁻¹ acetochlor and incubated at 30 °C for approximately 4 days. The number of colonies with transparent halos on the plates was counted, and the survival ratio of bacteria (SR, viable cell number ratio—cfu ml⁻¹ broth or cfu g⁻¹ powder—before and after

storage) was estimated to evaluate the method. To evaluate the powder degrading ability against acetochlor, bacteria powder before and after the storage was resuspended in 30 ml MSM to a final cell density of 1.0 (OD₆₀₀) and the acetochlor was added to a final concentration of 200 mg l⁻¹. The mixture was incubated under aerobic conditions on a rotary shaker at 30 °C, 180 r min⁻¹ for 48 h. The substrate concentration was determined by HPLC analysis as described by Chen et al. (2014).

Bacteria survival ratio (SR) =

$$\frac{\text{Viable cell number after stored at 180 d (cfu ml}^{-1} \text{ or cfu g}^{-1}\text{)}}{\text{Initial viable cell number (cfu ml}^{-1} \text{ or cfu g}^{-1}\text{)}} \quad (1)$$

Results

Optimization of submerged culture conditions for the biomass of liquid inoculant using response surface methodology (RSM)

Based on the results of PB design, the regression analysis and coefficients were calculated using Design Expert 8.0.6 software (Table 4). For example: factor G (K₂HPO₄), with the partial regression coefficient 0.47 and standard error 0.12, influence level *E* (*G*) equal to 0.94, showed that K₂HPO₄ had a positive effect on the biomass of strain DC-6. Thus, we improved *G* value in the subsequent factor optimization tests. The contribution value of *G* was 45.09%, that was significantly higher than the corresponding values of *A*, *C*, *D*, *E*, *F*, *H*, *J*, and *I* corresponding values. The results also showed that the contribution values of *B* (pH), *G* (K₂HPO₄), and *K* (yeast extract/(NH₄)₂SO₄) were 12.69, 45.09 and 17.86%, respectively, whereas the virtual factor *I* was only 6.01%. These results indicated that the linear model was applicable. By a multivariate regression analysis, a second-order model equation of

Table 4 Partial regression coefficients and significance analysis

Factor	Regression coefficient	Standard error	<i>E</i> (t)	Contribution value (%)	Sum of squares	Whether significant
Intercept	1.96	0.12				No
<i>A</i>	0.093	0.12	0.19	1.76	0.10	Yes
<i>B</i>	0.25	0.12	0.50	12.69	0.75	No
<i>C</i>	0.10	0.12	0.21	2.18	0.13	No
<i>D</i>	0.12	0.12	0.24	2.94	0.17	No
<i>E</i>	0.15	0.12	0.30	4.64	0.28	No
<i>F</i>	−0.026	0.12	−0.052	0.14	8.246e−003	No
<i>G</i>	0.47	0.12	0.94	45.09	2.68	Yes
<i>H</i>	0.021	0.12	0.042	0.089	5.277e−003	No
<i>I</i>	0.17	0.12	0.34	6.01	0.36	No
<i>J</i>	−0.18	0.12	−0.36	6.61	0.39	No
<i>K</i>	−0.30	0.12	−0.59	17.86	1.06	Yes

fermentation through strain DC-6 was fitted using the following equation, where *Y* was the predicted biomass in real value:

$$Y = 1.96 + 0.25B + 0.47G - 0.30K \quad (2)$$

The *P* value (Prob > *F*) was 0.0078, suggesting that the equation was significant. Based on the data obtained from the PB design study, three significant variables were further investigated through BBD design to identify optimum levels and interactions. Experimental design including strain DC-6 biomass measured and estimated values are shown in Table 3. Fermentation under the optimal conditions was simulated using this model. The full quadratic equation was the following:

$$\begin{aligned} \text{Biomass (g l}^{-1}\text{)} = & 2.86 + 0.004325X_1 + 0.004719X_2 \\ & - 0.036X_3 + 0.008651X_1X_2 \\ & + 0.013X_1X_3 + 0.003932X_2X_3 \\ & - 0.52X_1^2 - 0.16X_2^2 - 0.11X_3^2 \end{aligned} \quad (3)$$

where *X*₁, *X*₂, and *X*₃ are yeast extract/(NH₄)₂SO₄ (mol/mol, 3/1) (g l^{−1}), pH and K₂HPO₄ (g l^{−1}), respectively. According to the analysis of variance (ANOVA), general regression relations of the regression model reached extremely significant levels and the regression equation fitting *R*² was 0.9942, meaning a strong correlation; correction Radj² decision coefficient was 0.9866, meaning that 98.66% of the data in screening tests can be explained, and *P* < 0.001 meaning that this model was fitting the strain DC-6 biomass during the fermentation very well. These results illustrated that the model could fully optimize the strain DC-6 biomass production process.

Then three-dimensional response surface plots and respective contour plots are shown in Fig. 1. According to the graphic analysis, interactions between two factors and response surface peak point indicated the existence of a maximum biomass value within the scope of the selected factors.

The validation experiment conducted under optimum conditions was performed as follows: 25 g l^{−1} yeast extract/(NH₄)₂SO₄ (mol/mol, 3/1), 15 g l^{−1} glucose, 0.5 g l^{−1} K₂HPO₄, 0.5 g l^{−1} KH₂PO₄, 1.0 g l^{−1} NaCl, 0.5 g l^{−1} MgSO₄, pH 7.0, medium volume 30/250 (v/v, %), inoculum amount 7% (v/v) at 30 °C and 180 r min^{−1}. The verification experiment showed a 0.22-fold increase in the initial biomass of 2.35 g l^{−1} broth cultivated under the original non-optimized conditions such as LB broth with medium volume 100/250 (v/v, %) and inoculum amount 2% (v/v) at 30 °C and 180 r min^{−1}. The enhancement of biomass before and after fermentation optimization is shown in Fig. 2. Thus, the fermentation under the optimal conditions fitted the model and greatly improved the biomass.

Fed-batch fermentation using a 7.5-l bioreactor

The growth process of strain DC-6 in fermentation was studied with an initial glucose concentration of 15 g l^{−1} according to optimized results in shake flasks (Fig. 3). Low biomass with low glucose consumption was obtained during the first 12 h. Then, glucose levels began to fall progressively from 12 to 36 h as the cell population began to increase. Glucose concentration decreased to only 0.36 g l^{−1} that corresponded to less than 3% of the initial concentration at 36 h. At the same time, the biomass reached more than 4 g l^{−1} broth which was a value near to the maximum. Then, due to

Fig. 1 Corresponding contour plots and response surface plots for the optimization of strain DC-6 biomass. **a** Yeast extract/ $(\text{NH}_4)_2\text{SO}_4$ and pH; **b** Yeast extract/ $(\text{NH}_4)_2\text{SO}_4$ and K_2HPO_4 ; **c** pH and K_2HPO_4

Fig. 2 Biomass curve before and after fermentation optimization. (■) Strain DC-6 biomass change before fermentation optimization; (□) Strain DC-6 biomass change after fermentation optimization. Data are expressed as mean $\pm$ standard deviation for treatments in triplicate

the depletion of carbon source, the cultures reached the stationary phase with a maximum biomass of 5.13 g l^{-1} broth, which was 2.78 times more than the biomass reached by the shake flask culture. After fermentation, 98.41% glucose was consumed, and the corresponding number of living bacteria reached $2.87 \times 10^9 \text{ cfu ml}^{-1}$ broth.

Bacteria powder preparation by spray drying

Orthogonal test was conducted and the result of the spray drying of liquid inoculant was analyzed by the extreme difference as shown in Table 5. The experimental results from Table 5 were further treated by the range analysis to determine the most significant parameters for the number

Fig. 3 Curve of biomass growth and glucose consumption. (■) Concentration change for 15 g l^{-1} glucose; (□) Strain DC-6 biomass increase with glucose consumption. Data are expressed as mean $\pm$ standard deviation for treatments in triplicate

of viable cells in powder prepared by spray drying. Based on the results, we could conclude that the primary and secondary factors affecting the process were inlet air temperature > feed speed > airflow rate. The inlet air temperature was the most significant factor to affect the number of viable cells and the influence of feed speed was also significant. Thus, the run A2B1C2 was selected as the best group for spray drying. Under the optimized condition, concentrated liquid inoculant was made into 5 g bacteria powder with high activity of $2.11 \times 10^{12} \text{ cfu g}^{-1}$ powder, which was 2.43 times as the lowest run.

Performance tests of liquid inoculant and powder

Figure 4b shows the good fineness of bacteria powder with particles size of approximately $10 \mu\text{m}$. The morphology of

Table 5 Orthogonal array design for spray drying of strain DC-6

Run	A Inlet air temperature (°C)	B Hot air flow rate (m ³ min ⁻¹)	C Feed speed (ml min ⁻¹)	D Black	Viable cell numbers (10 ¹² cfu g ⁻¹ powder)
1	1 (120)	1 (0.4)	1 (10)	1	1.89
2	1 (120)	2 (0.6)	2 (15)	2	1.94
3	1 (120)	3 (0.8)	3 (20)	3	1.84
4	2 (140)	1 (0.4)	2 (15)	3	2.11
5	2 (140)	2 (0.6)	3 (20)	1	1.06
6	2 (140)	3 (0.8)	1 (10)	2	1.37
7	3 (160)	1 (0.4)	3 (20)	2	0.87
8	3 (160)	2 (0.6)	1 (10)	3	0.91
9	3 (160)	3 (0.8)	2 (15)	1	0.89
k1	1.89	1.62	1.39		
k2	1.51	1.30	1.65		
k3	0.89	1.36	1.26		
R	1.00	0.32	0.39		

Fig. 4 Microphotographs of strain DC-6 in liquid inoculant (a) and bacteria powder (b)

strain DC-6 after spray drying under the best conditions showed a round or oval particle shape with a smooth surface.

Since the shelf life is an important index to evaluate the quality and performance of microbial inoculant, we compared the preservation characteristics of liquid inoculant and bacteria powder. Under general preservation conditions, viable cell number of liquid inoculant rapidly decreased after 30-days and only 1.14×10^7 cfu ml⁻¹ broth could be found after 180-days storage. On the other hand, the optimized bacteria powder remained 1.91×10^{12} cfu g⁻¹ after 180-days storage and the SR was still at 90.5% (Fig. 5), suggesting that only few bacteria in the powder died during the storage. Furthermore, both the 180-days stored powder and the original powder showed the same degradation ability, which could completely degrade 200 mg l⁻¹ acetochlor within 48 h (data not shown).

Through the exploration strategy applied in this paper, we have successfully prepared an excellent microbial inoculant degrading pesticides, being useful for industrial production. Thus, the process-modeling was summarized in Fig. 6 as a reference for future studies on preparation of other microbial inoculant degrading pesticides.

Fig. 5 Viable cell number comparison between liquid inoculant and bacteria powder. Viable cell number in the liquid inoculant (■) and bacteria powder (▲) stored at room temperature. Each point represents the mean of three replicate measurements; standard error of the mean was less than 5%

Fig. 6 The process-modeling regarding the preparation of pesticide degrading microbial inoculant

Discussion

Submerged culture is a key technology to obtain high yield liquid inoculant (Jafarzade et al. 2013). Razak and Viswanath (2015) optimized the production of L-lysine using *Corynebacterium glutamicum* MH 20–22 B, which produced 26.34 g l^{-1} L-lysine in a 5-l bioreactor. However, few reports are available regarding optimization strategies for submerged fermentation (SmF) to increase biomass or enhance enzyme activity of pesticide degrading microbes. Meng et al. (2015) obtained the maximum production of chlorothalonil hydrolytic dehalogenase (Chd) from *B. subtilis* WB800 (pP43Chd). Chd activity increased from 6.80 to 14.50 U l^{-1} broth and the protein expression amount from 1.93 to $5.65 \text{ } \mu\text{g ml}^{-1}$ broth after optimization, but the optimized medium, containing 25 g l^{-1} tryptone, 31.2 g l^{-1} yeast extract, and 50 g l^{-1} glucose, was expensive. As regard wild pesticide degrading bacteria, Zhang et al. (2012) optimized the fermentation medium of *Acinetobacter* sp. DNS₃₂, an atrazine-degrading bacterium. The medium was composed of cheap corn flour 39.49 g l^{-1} and soybean flour 25.64 g l^{-1} . The optimized biomass in shake flasks reached $7.194 \times 10^8 \text{ cfu ml}^{-1}$ broth, which was lower than the optimized result in this study.

Several studies on *Sphingomonas* sp. fermentation were reported. The optimal fermentation medium for rhamsan gum production by *Sphingomonas* sp. CGMCC 6833 was studied and the highest rhamsan gum yield of $19.58 \pm 1.23 \text{ g l}^{-1}$ was obtained in the optimum medium containing 5.38 g l^{-1} soybean meal, $5.71 \text{ g l}^{-1} \text{ K}_2\text{HPO}_4$ and $0.32 \text{ g l}^{-1} \text{ MnSO}_4$. The yield was 42.09% higher than that of the initial medium ($13.78 \pm 1.38 \text{ g l}^{-1}$ broth) (Xu et al. 2015). This result was similar to our study, in which biomass could reach 2.86 g l^{-1} in shaking flask fermentation, which was 22% higher

than that of the initial medium (2.35 g l^{-1}), revealing that optimizing the fermentation medium of some *Sphingomonas* sp. may result in cheaper cost and higher yield. Thus, an optimized medium has the potential to be further applied for an industrial production.

Furthermore, the increase in nitrogen source for bacteria can be helpful (Bury et al. 1998). Jantzen et al. (2013) reported the growth enhancement of *Lactobacillus reuteri* when cultivated in watery 20% (w/v) whey solution with 0.5% (w/v) yeast extract in submerged slurry fermentation. In our study, the concentration of nitrogen source also acted as the most important factor for improving the yield of cells.

For stable degradation ability, high product yield, convenient transportation and improved shelf life, microbial powder preparation provides a potential choice to counter with these challenges. Spray drying technology provides many advantages such as simple operation, short time required, high yield and low cost over several drying methods (Gharsallaoui et al. 2007). It is also a preferred drying method for many thermally sensitive materials, such as milk and pharmaceuticals (Jafari et al. 2007; Vehring 2008; Huang et al. 2015). At present many reports are available on bacteria powder prepared by spray drying, focusing the attention on microbes such as *Mycobacteria*, *Bifidobacterium*, *Lactobacillus*, *Streptococcus thermophilus* and *Bacillus subtilis*. All these microbes showed a good tolerance to heat, and most of them were gram-positive. Jantzen et al. (2013) discovered that spray-drying process may offer an efficient option for industrial production of vital probiotics. In their study, *Lactobacillus reuteri* was directly spray dried and cell counts in the achieved product dropped by two orders of magnitude to approximately 10^7 after drying. However, few reports are available on pesticide-degrading microbes. The evaluation

of the best process conditions by orthogonal array design methods without addition of any protective agent or carrier can further enhance enzyme activity and lower cost (Lee et al. 2011). In comparison, in our study the bio-product decreased only 26.5% of 2.11×10^{12} cfu g⁻¹ powder after drying.

Microbes SR such as the one of lactic acid bacteria, was highly dependent on the spray drying process variables. It was reported that inlet air temperature was more detrimental to the total alive bacteria numbers (Bhandari et al. 1992). The process parameters were further optimized by RSM and the total alive bacteria numbers changed from 1.16×10^8 cfu g⁻¹ powder to 2.62×10^8 cfu g⁻¹ powder. The SR decreased with increased inlet air temperature, decreased atomization pressure and feed speed (Seth et al. 2016). Izadi et al. (2014) reported the highest SR of *Streptococcus thermophiles* at inlet temperature 150 °C, air flow 500 m³ h⁻¹, and feed speed 2 l h⁻¹. We also found that the inlet air temperature was the most significant factor to affect the number of viable cells and medium feed speed provided for higher SR. Bacteria powder reached a high activity of 2.11×10^{12} cfu g⁻¹ powder under the optimized condition (inlet air temperature 140 °C, feed speed of 15 ml min⁻¹, and a hot air flow rate of 0.4 m³ min⁻¹), which was 2.43 times as the lowest run.

Several spray-drying processes for dietary applications have been established using different polysaccharides and protein compounds to protect probiotics. Yu et al. (2010) studied gram-negative bacteria spray drying conditions by orthogonal array design and the SR was more than 84.57% when the inlet temperature was 160 °C, and feed speed of 12.5 ml min⁻¹. The protective agent was also added at a formula of arabic gum/malt dextrin 1/9 (m/m) in 50 ml of liquid nutrient medium.

The lower temperature may be beneficial for SR. Yu et al. (2010) studied the SR of gram-negative bacteria that were increased by 148% after storing in plastic film bag for 180-days under a temperature from 18 to 30 °C. Jantzen et al. (2013) studied product stability during storage at 4 °C and showed that cell counts of *Lactobacillus reuteri* in achieved products decreased from approximately 10⁷ after drying to 10⁶ after a storage period of 4 weeks. While in this study, the SR of bacteria powder remained at 90.5% even when stored at room temperature for 180-days, which could meet the standard of the microbial inoculants in agriculture and resulting more excellent than the results of previous studies.

In this study, the spray drying technology was first applied in gram-negative pesticide degradation bacteria without addition of any protective agent or carrier. Through the optimization of the parameters involved in the process, the number of viable cells was enhanced significantly, with improved shelf life, demonstrating that strain DC-6 was suitable for industrial production of bacteria powder, as well as the feasibility of the optimized conditions.

Conclusion

The RSM resulted at first in a 22% increase in biomass of acetochlor-degrading strain DC-6 under the optimized conditions. A biomass increase of 2.18 times with 14.58% shortened incubation period was furtherly obtained from the optimized medium in a 7.5-l bioreactor compared with flasks, while the number of viable cells in this liquid inoculant was difficult to maintain even for a shorter time. Finally, the bacteria powder was manufactured by spray drying of liquid inoculant, the SR of bacteria powder remained at 90.5% and removal ability still kept well even after 180-days storage at room temperature. The results showed that the microbial inoculant of strain DC-6 based on fermentation and spray drying could be feasible and used as a reference.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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