OPTIMIZATION OF MEDIUM COMPONENTS FOR CELL BIOMASS AND POLYHYDROXY BUTYRIC ACID PRODUCTION BY *Azotobacter vinelandii* MUTANT USING RESPONSE SURFACE METHODOLOGY

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Abstract

Polyhydroxy butyric acid (PHB) is a biodegradable and food-safe alternative to petroleum-based polymers. Using RSM approach, the interaction of sucrose, urea and K_2HPO_4 were investigated to determine the optimum medium compositions for cell biomass and PHB production by Azotobacter vinelandii mutant. Fifteen medium types were prepared and each contained different amount of sucrose, urea and K_2HPO_4. Analyses of cell biomass and PHB concentration were performed from day-2 until day-4 (3 days). Based on the biomass analysis, Medium 13 achieved the highest cell dry weight of 15.4 mg/mL on day-3. Medium 13 contained 0.5 g/L of urea, 0.1 g/L of K_2HPO_4 and 10 g/L of sucrose. For PHB production, Medium 11 achieved the highest PHB production on day-3 (3.7 mg/mL) and dropped to 1.3 mg/mL on day-4. Sample 11 contained 0.5 g/L of urea, 0 g/L of K_2HPO_4 and 20 g/L of sucrose. Sample 2 (1.0 g/L urea, 0.05 g/L K_2HPO_4 and 15 g/L sucrose) and 6 (1.0 g/L urea, 0.05 g/L K_2HPO_4 and 25 g/L sucrose) showed PHB production of >2.0 mg/mL on day-3 and persisted to day-4. Sample 3 (0.25 g/L urea, 0.2 g/L K_2HPO_4 and 15 g/L sucrose) achieved PHB production of >2.0 mg/mL only on day-4. All the other medium types showed PHB production of lower than 1.5 mg/mL throughout the experiment.

Keywords: *Azotobacter vinelandii* mutant, cell biomass, PHB, RSM

INTRODUCTION

Poly(3-hydroxybutyrate) (PHB) is a polymer with high potential for application as a degradable implant material and the simplest and most common group of polyhydroxy-alkanoates (PHA) (Freier et al. 2001). The advantages of PHB are it is truly biodegradable and is a food-safe alternative to petroleum-based polymers (Anbukarasu et al. 2015). It has potential for use in medical application and as food packaging materials (Zinn et al. 2001 and Kai & Loh 2014). However, disadvantages such as its brittleness and low flexibility, long degradation rate under physiological conditions and poor processability limit its potential for tissue engineering because of the crystalline nature of PHB (You et al. 2003).

In our study, response surface methodology (RSM) was used to determine the optimum medium components for the generation of cell biomass and PHB production by an *Azotobacter vinelandii* mutant. The RSM approach would enable better understanding of the interaction between the medium components (sucrose, dipotassium phosphate and urea), cell biomass and PHB production.

MATERIALS AND METHODS

A. vinelandii ΔAvin_16040 mutant was the bacterial strain used in this experiment. Bacterial inoculum was generated by aseptically transfer a single colony of *A. vinelandii* mutant to Burk's medium and grown for 3 days at 30ºC, 200 rpm. One percent (v/v) of the inoculum was transferred to modified Ashby-sucrose medium and the bacterial strain was incubated up to 4 days for cell growth and PHB production under the same condition. Our preliminary study indicated that sucrose, dipotassium phosphate (K_2HPO_4) and urea

were significant variables for cell biomass and PHB production. The weight of dry cell (g), OD_{600nm} and also OD_{235nm} (PHB analysis) were recorded at 2nd, 3rd and 4th days PHB analysis was performed by chloroform method according to Hiremath et al. (2015). RSM was conducted according to variable ranges and experimental design matrix as shown in Table 1 and Table 2, respectively.

Table 1. Experimental range and levels of the independent variables

Independent variables	Range and Levels				
	-α	-1	0	+1	+α
Urea (g/L)	0	0.25	0.5	1.0	2.0
K_2HPO_4 (g/L)	0	0.05	0.1	0.2	0.4
Sucrose (g/L)	10	15	20	25	30

Table 2. Experimental design matrix for cell biomass and PHB production

Sample	Urea (g/L)	K_2HPO_4 (g/L)	Sucrose (g/L)
1	0.25	0.05	15
2	1.0	0.05	15
3	0.25	0.2	15
4	1.0	0.2	15
5	0.25	0.05	25
6	1.0	0.05	25
7	0.25	0.2	25
8	1.0	0.2	25
9	0	0.1	20
10	2.0	0.1	20
11	0.5	0	20
12	0.5	0.4	20
13	0.5	0.1	10
14	0.5	0.1	25
15	0.5	0.1	25
15	0.5	0.1	25
15	0.5	0.1	25
15	0.5	0.1	25
15	0.5	0.1	25
15	0.5	0.1	25

RESULTS AND DISCUSSION

The effect of medium components on the mycelium biomass and PHB production was investigated. In total, fifteen medium compositions each contained differing amounts of sucrose (A), K_2HPO_4 (B) and urea (C).

Based on the results obtained, Medium 13 achieved the highest cell dry weight of 15.4 mg/mL on day-3. Medium 13 contained 0.5 g/L of urea, 0.1 g/L of K_2HPO_4 and 10 g/L of sucrose. While for PHB production, Medium 11 achieved the highest PHB production on day-3 (3.7 mg/mL) and dropped to 1.3 mg/mL on day-4. Medium 11 contained 0.5 g/L of urea, 0 g/L of K_2HPO_4 and 20 g/L of sucrose. Medium 2 (1.0 g/L urea, 0.05 g/L K_2HPO_4 and 15 g/L sucrose) and 6 (1.0 g/L urea, 0.05 g/L K_2HPO_4 and 25 g/L sucrose) showed PHB production of >2.0 mg/mL on day-3 and persisted to day-4. Sample 3 (0.25 g/L urea, 0.2 g/L K_2HPO_4 and 15 g/L sucrose) achieved PHB production of >2.0 mg/mL only on day-4. All the other medium types showed PHB production of lower than 1.5 mg/mL throughout the experiment.

Using RSM to study the interaction and contribution of medium components, we observed the affecting

factors for PHB production were A^2, B^2, AC, B^3, A^2B (3^{rd} day) and A, B, C^2, A^3, AC^2 (4^{th} day). Whilst, the affecting factors for cell biomass generation were B, C, A^2, C^2, AB, A^3 and B^2C.

CONCLUSION

In conclusion, the optimum growth conditions for maximum yields of *A. vinelandii* Δ*Avin_16040* cell biomass and PHB can be determined using the response surface methodology (RSM). The results suggested several interacting chemical factors which could affect the experimental models of PHB and cell biomass generation significantly.

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