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Optimization of medium composition for biomass production of recombinant *Escherichia coli* cells using response surface methodology

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Abstract

Factorial designs and second order response surface methodology (RSM) for medium optimization were employed for the growth of recombinant *Escherichia coli* cells carrying a plasmid encoding *Taq*I endonuclease as a part of the fermentation strategy for general recombinant protein production. The method used was effective in screening for nutritional requirements using limited number of experiments. The concentrations of carbon source (glucose), inorganic nitrogen ($(NH_4)_2HPO_4$), potassium (KH_2PO_4) and magnesium ($MgSO_4{\cdot}7H_2O$) sources in medium were changed according to the central composite rotatable design consisting of 29 experiments, and the biomass yield was calculated. The optimum medium composition was found to be 15 g L^{-1} glucose, 6.6 g L^{-1} $(NH_4)_2HPO_4$, 20.1 g L^{-1} KH_2PO_4 and 1.7 g L^{-1} $MgSO_4{\cdot}7H_2O$. The model prediction of 2.72 gDCW L^{-1} biomass at optimum conditions was verified experimentally as 2.68 gDCW L^{-1} which is much higher than any value obtained in initial experiments as well as in studies carried out previously. The correlation between biomass growth and *Taq*I endonuclease enzyme yield obtained under the same medium compositions was also analyzed.

Keywords: Medium design; Recombinant DNA; Enzyme production; Response surface methodology optimization

1. Introduction

Since the early development of recombinant DNA technology, *Escherichia coli* has been widely used as a host for high-level expression of recombinant proteins. Large-scale production of valuable proteins in this expression system is usually achieved using a two-stage process. In the first stage, cells are grown to a high cell density under favorable growth conditions in which protein synthesis is kept at minimum via tightly regulated promoter systems. This is followed by a second stage in which high-level expression of the recombinant protein is achieved upon induction.

E. coli grows in both rich complex organic media as well as in salt-based chemically defined media as long as an organic carbon source is provided. Through the type and concentration of ingredients used, cultivation medium composition directly dictates the amount of biomass produced, and therefore can dramatically influence the performance of microbial processes. Combinatorial interactions of medium components with the cell metabolism and the production of the desired compound are numerous and the optimum processes may be developed using effective experimental design procedures. Response surface methodology (RSM), which is a collection of statistical techniques for designing experiments, building models, evaluating the effects of factors and searching for the optimum conditions have successfully been used in the optimization of bio processes [1–7]. Gorret et al. [8] have reported the maximization of the biomass content of oil palm (*Elaeis guineensis*) and analyzed the effects of nitrogen source, inoculum size and conditioned medium on biomass production using RSM. In another study, Almeida e Silva et al. [9] used RSM in order to select nutrient levels for culturing *Paecilomyces variotii* in eucalyptus hemicellulosic hydrolyzate.

Previously, we have reported the extracellular production of *Taq*I restriction endonuclease of the thermophilic eubacterium *Thermus aquaticus* YT-1 as a fusion protein by recombinant *E.*

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coli cells [10]. Stability and extracellular secretion of the fusion protein were further improved by the co-expression of *Taq*I methylase [11]. Cytoplasmic expression of *Taq*I endonuclease in recombinant *E. coli* under the control of various promoters has also been reported [12–14]. We have also studied the optimization of medium composition for *Taq*I endonuclease production [15]. Our studies about the development of expression systems for high-level expression of recombinant proteins in *E. coli* clearly highlighted the need for optimization of medium composition for biomass growth prior to induction of protein synthesis. In addition, comparison of optimum medium compositions for biomass and protein production processes in order to investigate a possible correlation would lead to a better understanding of the whole system.

In the present study, the effects of medium components on biomass growth were investigated using RSM and compared with those of enzyme production. A correlation analysis was conducted to improve the production of a recombinant protein expressed under strongly controlled promoter.

2. Materials and methods

2.1. Cell cultivation

The plasmid (pTaqR + M) encoding *Taq*I restriction endonuclease under the control of the strong T7 RNA polymerase promoter and *Taq*I methylase under the control of constitutive tetracycline resistance gene promoter was constructed by Toksoy et al. [14].

Recombinant cells were cultivated in 1 L Erlenmeyer flasks containing 100 mL of medium of which the composition was specified according to the experimental design, in an orbital shaker at 37 °C and 180 rpm rotational speed. After 24 h of incubation, cells were harvested by centrifugation at 4000 rpm for 10 min. The biomass concentration in gDCW L^{-1} was determined by a gravitational method as described by Toksoy et al. [14].

2.2. Experimental design

The growth medium contained carbon source (glucose), inorganic nitrogen source ($(NH_4)_2HPO_4$), potassium source (KH_2PO_4), magnesium source ($MgSO_4{\cdot}7H_2O$) and various vitamins and trace metals. The concentrations of glucose, $(NH_4)_2HPO_4{\cdot}KH_2PO_4$ and $MgSO_4{\cdot}7H_2O$ were varied as parameter while the levels of vitamins and trace metal solutions were kept constant as previously described [14].

A 2^4 full factorial central composite design (CCD) with eight star points and five replicates at the center points was employed to fit a second order polynomial model. Twenty-nine experiments were required for this procedure as given in Table 1 [16]. The independent variables are coded for statistical calculations

Table 1
Experimental layout and biomass concentration results of recombinant *E. coli* cells in different media

Med No	Glucose	$(NH_4)_2HPO_4$	KH_2PO_4	$MgSO_4$	Biomass (gDCW L^{-1})	Yield (U gDCW^{-1}) × 10^{-6}
Ml	−1(10)	−1(2.5)	−1(10)	−1(0.5)	1.400	98.6
M2	1(30)	−1(2.5)	−1(10)	−1(0.5)	1.313	122.8
M3	−1(10)	1(7.5)	−1(10)	−1(0.5)	1.843	46.1
M4	1(30)	1(7.5)	−1(10)	−1(0.5)	1.799	71.9
M5	−1(10)	−1(2.5)	1(20)	−1(0.5)	1.167	42.7
M6	1(30)	−1(2.5)	1(20)	−1(0.5)	1.597	40.4
M7	−1(10)	1(7.5)	1(20)	−1(0.5)	1.199	28.2
M8	1(30)	1(7.5)	1(20)	−1(0.5)	1.429	55.2
M9	−1(10)	−1(2.5)	−1(10)	1(1.5)	1.237	81.3
M10	1(30)	−1(2.5)	−1(10)	1(1.5)	1.199	57.6
M11	−1(10)	1(7.5)	−1(10)	1(1.5)	1.722	46.9
M12	1(30)	1(7.5)	−1(10)	1(1.5)	1.419	35.6
M13	−1(10)	−1(2.5)	1(20)	1(1.5)	1.875	45.8
M14	1(30)	−1(2.5)	1(20)	1(1.5)	1.472	7.1
M15	−1(10)	1(7.5)	1(20)	1(1.5)	2.500	69.1
M16	1(30)	1(7.5)	1(20)	1(1.5)	2.014	66.4
M17	0(20)	0(5)	0(15)	0(1)	1.855	49.4
M18	0(20)	0(5)	0(15)	0(1)	2.007	47.2
M19	0(20)	0(5)	0(15)	0(1)	2.046	41.7
M20	0(20)	0(5)	0(15)	0(1)	1.883	40.8
M21	0(20)	0(5)	0(15)	0(1)	1.783	39.2
M22	1.4(30)	0(5)	0(15)	0(1)	2.181	48.5
M23	−1.4(6)	0(5)	0(15)	0(1)	1.850	97.3
M24	0(20)	−1.4(1.5)	0(15)	0(1)	1.640	128.4
M25	0(20)	1.4(8.5)	0(15)	0(1)	2.335	39.1
M26	0(20)	0(5)	−1.4(8)	0(1)	1.705	55.1
M27	0(20)	0(5)	1.4(22)	0(1)	2.202	31.4
M28	0(20)	0(5)	0(15)	−1.4(0.3)	2.066	1.9
M29	0(20)	0(5)	0(15)	1.4(1.7)	2.243	20.6

Numbers in parenthesis represent the real concentrations of each medium component in g L^{-1}.