Available online at www.sciencedirect.com
ScienceDirect
Bioresource Technology 99 (2008) 7875–7880
BIORESOURCE TECHNOLOGY

Short Communication

Use of response surface optimization for the production of biosurfactant from *Rhodococcus* spp. MTCC 2574

Snehal R. Mutalik [a], Bhalchandra K. Vaidya [a], Renuka M. Joshi [a], Kiran M. Desai [b], Sanjay N. Nene [a,*]

[a] *Biochemical Engineering Department, Chemical Engineering and Process Development Division, National Chemical Laboratory, Pune 411008, India*
[b] *Food Engineering and Technology Department, Institute of Chemical Technology, Matunga, Mumbai 400019, India*

Received 21 September 2007; received in revised form 21 February 2008; accepted 21 February 2008
Available online 3 June 2008

Abstract

The production of biosurfactant from *Rhodococcus* spp. MTCC 2574 was effectively enhanced by response surface methodology (RSM). *Rhodococcus* spp. MTCC 2574 was selected through screening of seven different *Rhodococcus* strains. The preliminary screening experiments (one-factor at a time) suggested that carbon source: mannitol, nitrogen source: yeast extract and meat peptone and inducer: *n*-hexadecane are the critical medium components. The concentrations of these four media components were optimized by using central composite rotatable design (CCRD) of RSM. The adequately high R^2 value (0.947) and F score 19.11 indicated the statistical significance of the model. The optimum medium composition for biosurfactant production was found to contain mannitol (1.6 g/L), yeast extract (6.92 g/L), meat peptone (19.65 g/L), *n*-hexadecane (63.8 g/L). The crude biosurfactant was obtained from methyl *tert*-butyl ether extraction. The yield of biosurfactant before and after optimization was 3.2 g/L of and 10.9 g/L, respectively. Thus, RSM has increased the yield of biosurfactant to 3.4-fold. The crude biosurfactant decreased the surface tension of water from 72 mN/m to 30.8 mN/m (at 120 mg L^{-1}) and achieved a critical micelle concentration (CMC) value of 120 mg L^{-1}.

Keywords: Biosurfactant; *Rhodococcus* spp.; Response surface methodology; Medium optimization

1. Introduction

Biosurfactants or microbial surfactants are surface-active biomolecules produced by a variety of microorganisms. Biosurfactants are receiving considerable attention due to their unique properties such as higher biodegradability, lower toxicity, and greater stability towards temperature and pH (Mukherjee et al., 2007). In the past few decades, biosurfactants have been identified for commercial importance, specifically, in the field of oil recovery (Ivshina et al., 1998); secondary or tertiary environmental bioremediation (Christofi and Ivshina, 2002); pharmaceuticals (Rodrigues et al., 2006a); and food processing and cosmetics (Banat et al., 2000).

Members of the genus *Rhodococcus* are known to produce surface-active trehalose-lipids. Bell et al. (1998) have reported that some biosurfactants including those from rhodococci are more effective and efficient than many existing synthetic surface-active agents. The production of biosurfactant from different strains of *Rhodococcus* has been reported in the literature (Lang and Philp, 1998; Kuyukina et al., 2001; Pirog et al., 2004). The yield of biosurfactant varies considerably with the hydrocarbon which is used to induce the production of biosurfactant.

The potential of *Rhodococcus* biosurfactant in a variety of industrial applications has been proposed, but like other biosurfactants it is yet to achieve market penetration (Mukherjee et al., 2007). *Rhodococcus* biosurfactants have not achieved significant market share because of their high cost as compared to synthetic surfactants. Thus, to compete with the large-scale production of synthetic surfactants from

* Corresponding author. Tel.: +91 20 25902347; fax: +91 20 25902612.
E-mail address: sn.nene@ncl.res.in (S.N. Nene).

0960-8524/$ - see front matter © 2008 Published by Elsevier Ltd.
doi:10.1016/j.biortech.2008.02.027

hydrocarbon feedstocks, it is desirable to enhance the production of biosurfactant by *Rhodococcus*. One of the methods of achieving the above objective is the selection of appropriate media components and optimal culture conditions for maximum biosurfactant productivity.

The limitations of classical method of media optimization can be overcome by the application of statistical based approach (Lotfy et al., 2007; Tanyildizi et al., 2005). RSM, an extensively used statistical technique for media optimization, is a collection of statistical techniques which uses design of experiments (DoE) for building models, evaluating the effects of factors and searching for the optimum conditions. (Rodrigues et al., 2006b).

In the present work, we have used a central composite rotatable design (CCRD) of response surface methodology for media optimization to enhance biosurfactant production by *Rhodococcus* spp. MTCC 2574.

2. Methods

2.1. Materials

All bacterial growth media components were purchased from Hi-Media, India. All other chemicals were of analytical grade procured from S.D. Fine Chemicals, India.

2.2. Microorganism and growth conditions

The seven microbial strains namely *Rhodococcus* spp. MTCC 2574, 2678, 2683 and *Rhodococcus erythropolis* MTCC 1526, 1548, 2794, 3951 were procured from MTCC-Chandigarh (India).

All *Rhodococcus* strains were maintained on nutrient agar (beef extract 10 g/L, NaCl 5 g/L, peptone 10 g/L, agar 20 g/L, pH 7.0–7.5). The liquid fermentation medium used for batch culture experiments contained ($g\,L^{-1}$) glucose (10), yeast extract (3), meat peptone (7.5), Na_2HPO_4 (4.0), KH_2PO_4 (2.0), $MgSO_4 \cdot 7H2O$ (0.2), $CaCl_2 \cdot 2H_2O$ (0.02), ammonium ferric citrate (0.05), trace mineral solution (1 mL/L) [termed as medium A]. The composition of trace mineral medium was ($g\,L^{-1}$) H_3BO_3 (0.1), $MnCl_2 \cdot 4H_2O$ (0.1), $ZnSO_4 \cdot H_2O$ (0.1), $FeCl_3 \cdot 6H_2O$ (0.1), $CaCl_2 \cdot 2H_2O$ (1), $CuCl_2 \cdot 2H_2O$ (0.05). Five milliliter of inoculum was transferred to 45 mL of liquid fermentation medium contained in a 250 mL Erlenmeyer flask and incubated for 36 h at 30 °C on rotary shaker at 200 rpm.

2.3. Selection of optimum nitrogen source, carbon source and inducer

The organic nitrogen source (yeast extract and meat peptone) from Medium A was replaced by inorganic nitrogen sources (namely urea, ammonium sulphate and ammonium phosphate) at equivalent nitrogen level. To evaluate the optimum carbon source, glucose was replaced by an equivalent amount of different carbon sources namely glycerol, sucrose, sorbitol and mannitol. Seven inducers (3% v/v each) were screened to evaluate the corresponding enhancement in biosurfactant production. Biosurfactant production was calculated in terms of emulsification index (% EI_{24}) as described in Section 2.7.1.

2.4. Biosurfactant profile

The cell growth and biosurfactant production were simultaneously studied to establish the biosurfactant profile. Cells of *Rhodococcus* spp. MTCC 2574 were grown on liquid medium containing ($g\,L^{-1}$) mannitol (10.12), yeast extract (3), meat peptone (7.5), Na_2HPO_4 (4.0), KH_2PO_4 (2.0), $MgSO_4 \cdot 7H_2O$ (0.2), $CaCl_2 \cdot 2H_2O$ (0.02), ammonium ferric citrate (0.05), trace mineral solution (1 mL/L) and *n*-hexadecane (3% v/v) (termed as medium B). The samples were aseptically removed at a regular interval of 4 h up to 48 h and analyzed for optical density (600 nm), % EI_{24} and % hydrophobicity.

2.5. Experimental design and data analysis

To examine the combined effect of four different medium components (mannitol, yeast extract, meat peptone and *n*-hexadecane) on biosurfactant production by *Rhodococcus* spp. MTCC 2574, 30 experiments were performed in duplicate. The value of the dependent response (% EI_{24}) was the mean of two replications. The second-order polynomial coefficients were calculated and analyzed using the trial version of 'Design Expert' software (Version 6.0, Stat-Ease Inc., USA).

2.6. Extraction of biosurfactant

The extraction of biosurfactant was done by using methyl *tert*-butyl ether (MTBE) as described earlier by Kuyukina et al. (2001). The product was thoroughly washed thrice with petroleum ether to remove residual *n*-hexadecane to obtain crude biosurfactant. The crude biosurfactant was finally dried by lyophilization.

2.7. Analytical methods

2.7.1. Emulsification Index (% EI_{24})

Emulsification Index (% EI_{24}) was used to quantify the biosurfactant produced by *Rhodococcus* cells (Fleck et al., 2000). The values of % EI_{24} were determined as described earlier by Nitschke and Pastore (2006). The percent ratio of the height of emulsified zone to total height after 24 h gives 'emulsification index' (% EI_{24}) as given in Eq. (1)

$$\%\ EI_{24} = \frac{\text{Height of emulsified zone}}{\text{Total height of liquid(sum of aqueous, oil and emulsified zone)}} \times 100 \quad (1)$$