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Optimization of biosurfactant production by *Bacillus brevis* using response surface methodology

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ABSTRACT

The present study aims to evaluate and validate a statistical model for maximizing biosurfactant productivity by *Bacillus brevis* using response surface methodology. In this respect, twenty bacterial isolates were screened for biosurfactant production using hemolytic activity, oil spreading technique, and emulsification index (E24). The most potent biosurfactant-producing bacterium (*B. brevis*) was used for construction of the statistical response surface model. The optimum conditions for biosurfactant production by *B. brevis* were: 33 °C incubation temperature at pH 8 for 10 days incubation period and 8.5 g/L glucose concentration as a sole carbon source. The produced biosurfactant (BS) (73%) exhibited foaming activity, thermal stability in the range 30–80 °C for 30 min., pH stability, from 4 to 9 and antimicrobial activity against (*Escherichia coli*). The BS gave a good potential application as an emulsifier.

1. Introduction

Surfactants are widely used for industrial, agricultural, food, cosmetics and pharmaceutical applications. Most of these compounds are chemically synthesized and potentially causing environmental and toxicological problems [19,30]. Therefore, microbial-derived surface-active compounds attract attention essentially due to their low toxicity, biodegradable nature [32,34], better environmental compatibility "Green Technology" and easily operated [5]. Recently, biosurfactants received much attention in nano biotechnology criteria [33,26]. Furthermore, biosurfactants have antibacterial (inhibition activity of cell wall synthesis) [14,27], antifungal and antiviral properties. They inhibit tumor growth and toxic effects, they also are immune stimulant and cell lysis (haemolysis) [4], they are less allergic, can be used as adhesive agents also, in vaccines and gene therapy [11]. Biosurfactants can be found in detergents, laundry formulations, household cleaning products, herbicides or pesticides, bioremediation, agriculture, textile, paper, petroleum industries, pharmaceutical and food-processing industry [6,24]. Also, in enzyme stimulation and bio-regulatory effects [25]. They are important in plant pathogenicity, effective on migration of human neutrophils, respiratory action (anti-asthma activity) and food digestion [20], paint, cement, beer, beverages hygiene and cosmetics [23]. Furthermore, biosurfactants are usually effective at extreme environmental conditions and can be produced from renewable resources [9].

In this investigation, the power of response surface method using central composite design (CCD) had been explored to optimize biosurfactant production by *Bacillus brevis*. Therefore, in this study, the effect of temperature (A), pH (B), incubation period (C) and glucose concentration (D) for maximizing biosurfactant production by *B. brevis* using central composite design had been evaluated and validated, experimentally.

2. Materials and methods

2.1. Microorganism and culture conditions

Different samples were collected from oil contaminated soil and sediment of mangrove trees. To isolate bacteria, these sample were cultured on the following medium [3] (g/L): NaNO3 (2.0), KCl (0.5), Na2HPO4H2O (1.0), KH2PO4 (1.0), CaCl2 (0.025); MgSO4 (0.1), FeSO4.7·H2O (0.001) and 2 ml/L trace element solution containing the following ingredients (mg/L): FeCl3·6H2O (60), ZnSO4·7H2O (600), MnSO4·H2O (200), CuSO4·5H2O (590), CoCl2·6H2O (60). The pH of the medium was adjusted to 7.0 and sterilized by autoclaving at 121 ◦C for 20 min. A potent biosurfactant-producing bacterium has been isolated from the sediment of mangrove trees (Makadi vallige, Hurghada region, Egypt), purified and characterized. This isolate has been identified

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Table 1
Central Composite design runs showing factors and their levels (based on actual value).

Run	Run type	(*A*) Incubation temperature (°C)	(*B*) pH	(*C*) Incubation period (days)	(*D*) Glucose concentration (g/L)
1, 26, 59, 71	Factorial	25	6	3	5
2, 20, 94, 100	Factorial	35	6	7	5
3, 9, 34, 37, 42, 50, 57, 58, 65, 70, 84, 95	Center	30	7	5	10
4, 19, 55, 81	Factorial	35	6	3	5
5, 47, 88, 91	Factorial	25	8	7	5
6, 15, 68, 108	Factorial	25	8	7	15
7, 38, 83, 96	Factorial	25	6	7	5
8, 52, 63, 99	Axial	30	5	5	10
10, 54, 79, 90	Axial	30	7	1	10
11, 21, 62, 92	Axial	20	7	5	10
12, 13, 93, 78	Factorial	25	6	7	15
14, 29, 101, 102	Factorial	35	8	3	5
16, 46, 77, 98	Factorial	35	6	3	15
17, 22, 60, 64	Factorial	35	6	7	15
18, 43, 76, 103	Factorial	35	8	3	15
23, 32, 56, 61	Axial	30	7	5	0
24, 33, 87, 104	Factorial	35	8	7	5
25, 31, 67, 89	Factorial	25	8	3	5
27, 30, 74, 105	Factorial	25	6	3	15
28, 53, 82, 97	Axial	30	9	5	10
35, 40, 75, 107	Axial	30	7	9	10
36, 41, 73, 86	Axial	40	7	5	10
39, 51, 66, 80	Factorial	25	8	3	15
44, 45, 69, 85	Factorial	35	8	7	15
48, 49, 72, 106	Axial	30	7	5	20

based on 16S-rRNA. The pure culture was preserved at (4 °C) and subculturing was done every month.

3. Biosurfactant productivity tests

3.1. Hemolytic activity

A pure culture of each bacterial isolate was streaked on the freshly prepared blood agar and incubated at 37 °C for 48–72 h. Results were recorded based on the type of clear zone observed [21,35].

3.2. Oil spreading method

Oil spreading technique was carried out according to the method described by Satpute et al. [31]. Briefly, 50 mL of distilled water was added to the Petri plate followed by addition of 100 μL of olive oil to the surface of the water. Then, 10 μL of cell-free culture broth was dropped on the crude oil surface. The diameter of the clear zone on the oil surface was measured and compared to 10 μL of distilled water as a negative control.

3.3. Emulsification activity (E24)

The emulsification activity was measured using the method described by Plaza et al. [22]. About 2 mL of olive (crude oil) and 2 mL of cell-free medium (supernatant) were inoculated to a test tube and homogenized by vortexing at high speed for 2 min. After 24 h, the emulsification activity was calculated using following formula:

E24 (%) = total height of the emulsified layer/total height of the liquid layer [15].

3.4. Identification of bacterial isolate

The most efficient biosurfactant producer bacterial isolate was then identified as *B. brevis* using 16S rRNA analysis Procedure, which has been performed at Macrogen company (Korea) and used for the current investigation.

3.5. Foam height analysis

Foaming ability was determined according to Abou seoud et al. [1]. *B. brevis* was grown in 250 mL Erlenmeyer flask, containing 50 mL of nutrient broth medium. The flask was incubated at 33 °C on a shaker incubator (200 rpm) for 96 h. Foam activity was detected as the duration of foam stability, foam height and foam shape in the graduated cylinder.

Table 2
Screening for biosurfactant producing isolates by preliminary and complementary screening methods.

Isolate No.	Hemolytic activity	Oil displacement area (cm^2)	Emulsification index (%)
1	–	2.2	0
2	–	1.8	0
3	+	4.4	0
4	–	1.4	6.4
5	+	4.6	0
6	+	4.2	0
7	+++++	28.2	46.6
8	+	3.6	0
9	++	2.8	24.2
10	+	2.6	20.2
11	+++	12.2	26.4
12	++	2.2	22.6
13	+	3.1	18.8
14	+++	16.8	24.4
15	++	3.6	22.6
16	++	2.8	23.6
17	++	2.9	24.3
18	+	1.4	16.6
19	++	2.8	22.4
20	++	2.4	22.6