

Optimization of biosurfactant production from *Vibrio* sp. BSM-30 isolated in tropical waters

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Abstract. The strain BSM-30 (*Vibrio* sp.), isolated from Chinese tropical waters, could be a biosurfactant producing bacteria according with results obtained by the oil spreading method. The culture conditions for biosurfactant production were tested respectively such as inoculation (2%,6%,10%,14% as setting), shaking speed(120 r/min,150 r/min,180 r/min as setting), temperature (25°C,30°C,35°C as setting), pH (7,8,9 as setting), salinity (1.5%, 2.5%, 3.0%, 4.5%, 5.5% as setting), which results showed that the best culture conditions for BS production were 10% inoculation quantity, 180 r/min, 25°C, pH 8, and 3.5% salinity. The optimization of carbon sources (20g/ of glucose, 20g/L of starch, 20g/L of paraffin oil 20g/L of diesel, 20g/L of oil as setting) and nitrogen sources (6g/L of NaNO₃,7.1g/L of KNO₃,5.6g/L of NH₄NO₃,9.3g/L of (NH₄)₂SO₄, 4.2g/L of CO(NH₂)₂ as setting) were also tested, which results showed that the best nitrogen source and carbon source were (NH₄)₂SO₄ and soluble starch.

1. Introduction

Biosurfactant (BS) are metabolites in the microorganism metabolism which reduce surface tension. Other properties associated with BS are increase of solubility, emulsifying, foaming, wetting, and dispersing. At present, most BS producing bacteria come from the oil polluted environment, and they can also be applied to petroleum hydrocarbon pollutant remediation in environment due to BS's properties such as non-toxic or low toxicity, readily biodegradable, environmentally friendly. [1, 2] Some scholars studies [3,4] showed that BS producing bacteria made very good effect of oil removing in oil sludge by washing directly with its bacterial liquid. The focus of this research is screening BS producing bacteria and the optimization of strain living conditions to prepare for later repair test of petroleum hydrocarbon pollutants.

2. Materials and method

2.1. Materials

Sampling



In May 2, 2015, seawater samples were obtained from Baishamen offshore, Haikou, China. Samples were below 0 °C during transportation.

Culture medium

Rich culture medium (RCM)[5]: Beef Extract 0.5%, Peptone 1%, NaCl 0.5%, Agar 1.5-2%, stale seawater 1L, pH 7.5, sterilized 20min under 121 °C.

Filtration culture medium: Rich culture medium containing 10ml oil for screening BS producing bacteria.

Bacterial fermentation medium (BFM) [6]: glucose 10g, NH₄Cl 1.5g, K₂HPO₄·12H₂O 3g, KH₂PO₄ 0.5g, CaCl₂ 0.01g, FeSO₄·7H₂O 0.015g, MnSO₄ 0.002g, CuSO₄·5H₂O 0.002g, stale seawater 1L, pH 7.5, sterilized 20min under 121 °C.

Nitrogen deficient medium: Bacterial fermentation medium without nitrogen compounds
 Carbon deficient medium: Bacterial fermentation medium without carbon compounds.

2.2. Method

2.2.1. Degradation bacterium enrichment. The seawater samples, which were diluted in gradient 10⁻¹, 10⁻², 10⁻³, 10⁻⁴, were spread (1 ml) on RCM. After culture, colonies were streaked for purification. All of single bacteria isolated were inoculated BFM (150r/min, 30 °C, 4-5d), [4] and from of them, the dominant surfactant bacterium were screened for BS production by the oil spreading method. [7~10] The bacteria BSM-30 selected in this research is a strain of *Vibrio* sp. by the molecular identification (RNAr 16r Sequencing).

2.2.2. Culture conditions optimization for BSM-30. The orthogonal method was used to optimize BSM-30 culture conditions with inoculum, temperature, pH, speed as factors. Results obtained are measured as bacterial growth (OD₆₀₀) and BS production (clearing zone diameter). Studied conditions for orthogonal design are shown in Table1 and Table2.[11,12]

Table1. The orthogonal test and the selection of level

Level	Factor			
	Inoculum /%	pH	T/°C	Speed r/min
1	2	7	25	120
2	6	8	30	150
3	10	9	35	180

Table2. The optimization of orthogonal experimental design

Sequence	Inoculum /%	pH	T/°C	Speed r/min
1	2	7	25	120
2	2	8	30	150
3	2	9	35	180
4	6	7	30	180
5	6	8	35	120
6	6	9	25	150
7	10	7	35	150
8	10	8	25	180
9	10	9	30	120

2.2.3. Nitrogen source optimization experiment. NaNO₃, KNO₃, NH₄NO₃, (NH₄)₂SO₄, CO(NH₂)₂ were used as nitrogen source of nitrogen deficient medium respectively (concentration of nitrogenous species are same as BFM), in which BSM-30 was continuously cultured (25 °C, 180 r/min, 108 h),

and then, the OD₆₀₀ incremental and the clearing zone diameter of BSM-30 fermentation liquid were measured.

2.2.4. Carbon source optimization experiment. Glucose, Starch, Paraffin oil, Diesel oil, Oil (from Fushan oilfield Hainan province, China) were used as carbon source of carbon deficient medium respectively (concentration of carbon species are same as BFM), in which BSM-30 was continuously cultured (25 °C, 180 r/min, 108 h), and then, the OD₆₀₀ incremental and the degreasing cycle of BSM-30 fermentation liquid were measured.

2.2.5. NaCl optimization experiment. Different NaCl concentrations (such as 1.5%, 2.5%, 3.0%, 4.5%, 5.5%) were given to bacterial fermentation medium respectively, in which BSM-30 was continuously cultured (25 °C, 180 r/min, 108 h), and then, the OD₆₀₀ incremental and the clearing zone diameter of BSM-30 fermentation liquid were measured.[13]

3. Results and Discussion

3.1. The growth curve and degreasing cycle

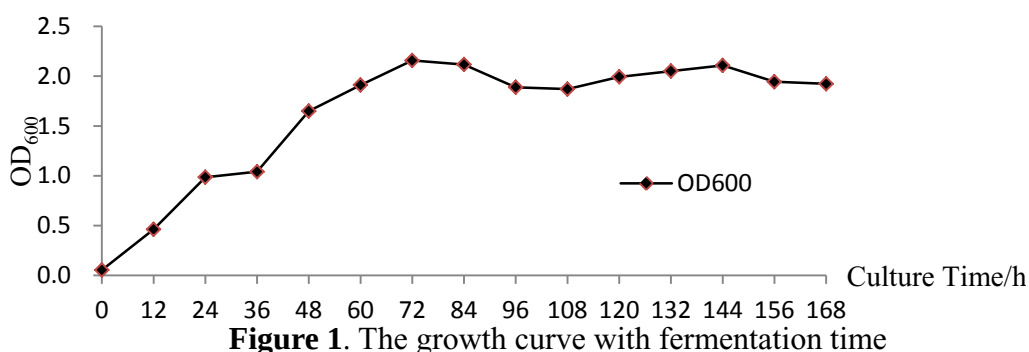


Figure 1. The growth curve with fermentation time

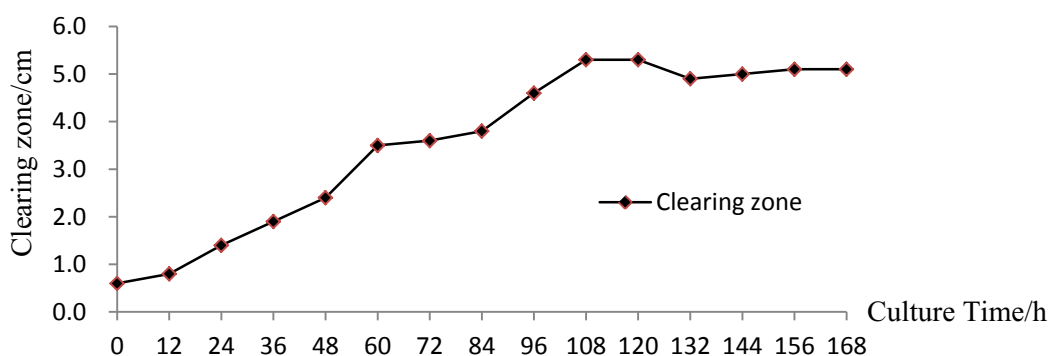


Figure 2. Clearing zone changes with fermentation time

The strain BSM-30 was grown rapidly till 72 h after inoculation, when its grown entered the stationary phase (Figure 1). But BS production of BSM-30 has a certain lag than bacterial growth, the clearing zone diameter of BSM-30 grown supernatant increased with time till to max 5.3 cm, which time is about 108 h, and then remained unchanged (Figure 2). So the 108 h is the optimum culture time when the aim is to obtain the biosurfactant of BSM-30 secretions.

Table 3. The result of orthogonal experimental optimization

Sequence	Inoculum /%	pH	T/°C	Speed r/min	Clearing zone /cm
1	2	7	25	120	3.9
2	2	8	30	150	3.9
3	2	9	35	180	5.5
4	6	7	30	180	6.0
5	6	8	35	120	1.8
6	6	9	25	150	2.9
7	10	7	35	150	2.8
8	10	8	25	180	6.3
9	10	9	30	120	5.0
T1	13.3	13.0	13.2	10.7	
T2	11.0	12.1	15.2	9.6	
T3	14.2	13.4	10.1	18.2	
t1	4.4	4.3	4.4	3.6	
t2	3.7	4.0	5.1	3.2	
t3	4.7	4.5	3.4	6.1	
R	1.0	0.5	1.7	2.9	

3.2. Culture conditions optimization

The Temperature and Speed have greater influence on clearing zone diameter of BSM-30 than other factors (the grey parts in table 3). The optimal culture conditions should be 10% inoculation quantity, pH 8, 25 °C, 180 r/min, while the clearing zone diameter can grow to 6.3 cm which increase 20.8 % more than the original one.

3.3. Nitrogen source optimization experiment

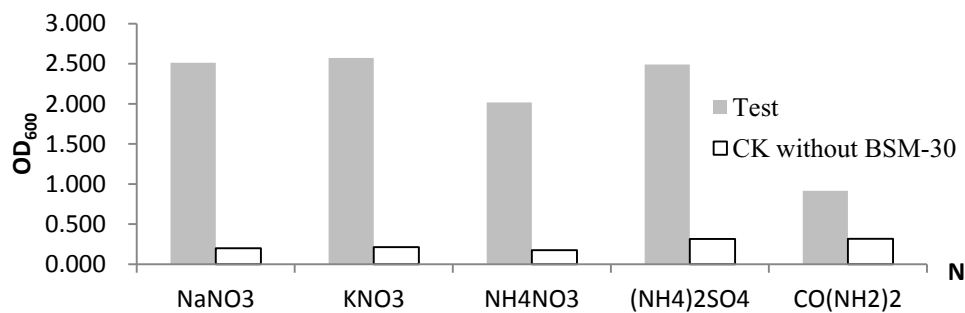


Figure 3. N optimization (OD)

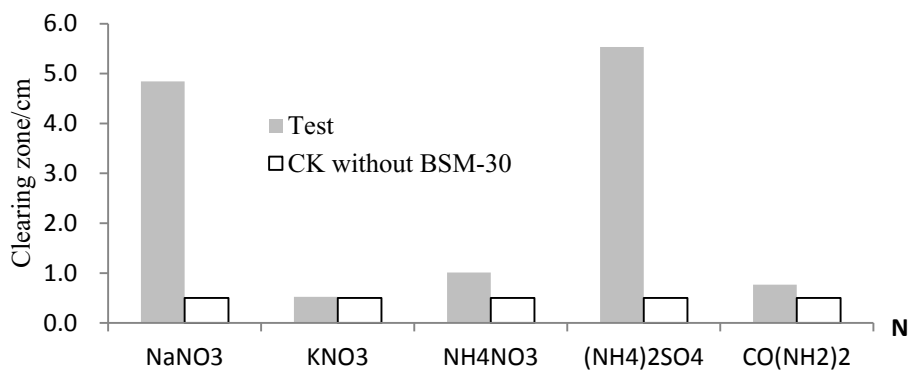


Figure 4. N optimization (Clearing zone)

The strain BSM-30 can get better growth whatever NaNO₃, KNO₃, NH₄NO₃, (NH₄)₂SO₄ were respectively used as nitrogen source (Figure 3), while the strain BSM-30 not only grow well but also could produce more biosurfactant when (NH₄)₂SO₄ was used as nitrogen source than others, which clearing zone diameter was big as 5.5 cm (Figure 4).

3.4. Carbon source optimization experiment

The test results showed that Glucose and Starch were more suitable for strain BSM-30 culture as carbon source (Figure 5), while although the strain BSM-30 showed moderate levels of proliferation when the petroleum hydrocarbons were used as carbon source, but their fermentation liquid had more biosurfactant than others for their clearing zone diameter even bigger (Figure 6).

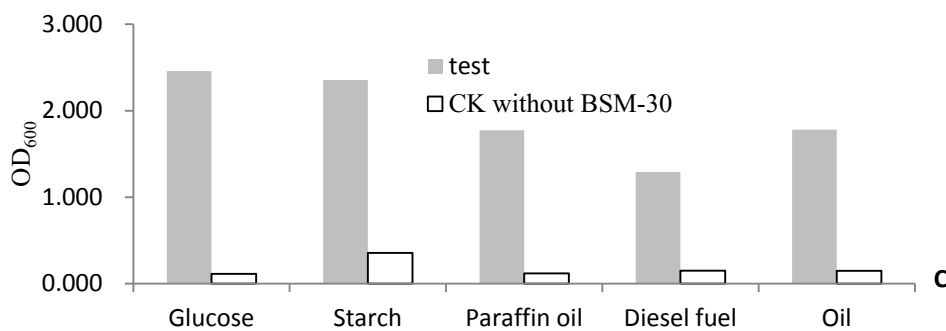


Figure 5. C optimization (OD)

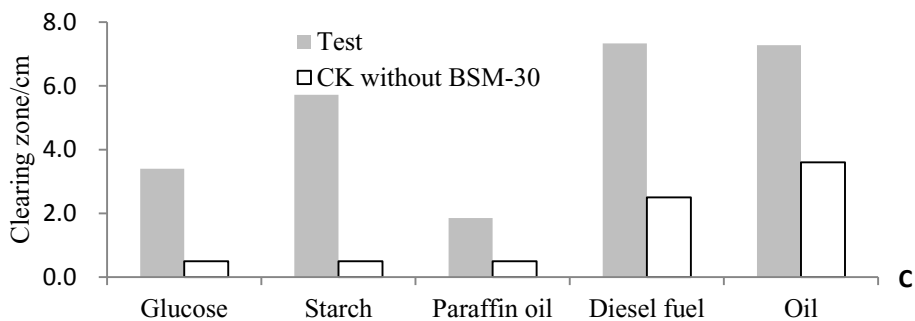


Figure 6. C optimization (Clearing zone)

3.5. NaCl optimization experiment

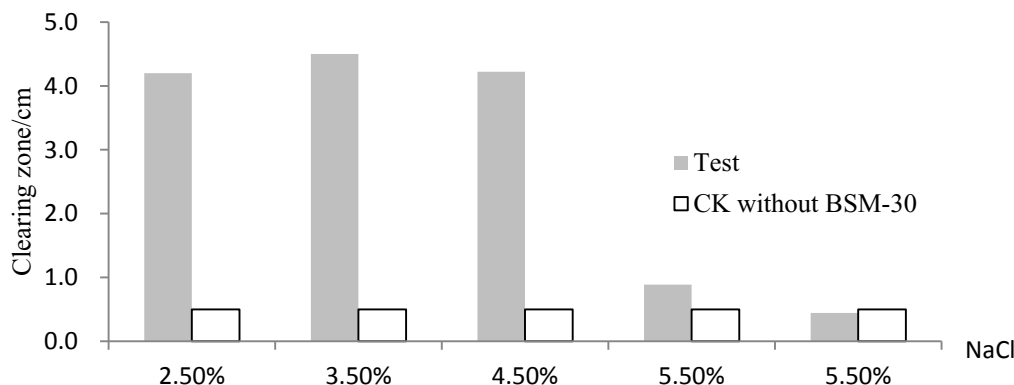


Figure 7. NaCl optimization (Clearing zone)

The salinity of about 3.5% was more suitable for the growth of strains, which is consistent with the mid latitude ocean salinity and the seawater salinity of strains screening (Figure 7), and the test results also showed that the salinity was suitable for biosurfactant secretion activity of the strain BSM-30.

4. Discussion

The results of this study showed that the optimum culture conditions of strain BSM-30 were 10% inoculation quantity, pH 8, 25 °C, 180 r/min, (NH₄)₂SO₄ as nitrogen source, soluble starch as carbon source and 3.5% salinity. Under these conditions, the proliferative activity of BSM-30 peaked at around 72 hours and surfactant secretion peaked at 108 hours, which described that BS production is a non-associated to bacterial growth., the restriction of those nutrition factors may be the reason of that. Although the strain BSM-30 showed moderate levels of proliferation when the petroleum hydrocarbons were used as carbon source, but their fermentation liquid supernatant produce more biosurfactant than others from their clearing zone diameter, which will be conducive to its application to assist petroleum hydrocarbon degrading strains in environment polluted by petroleum hydrocarbon. In addition, the growth and surfactant secretion of BSM-30 were not affected by the salinity of 3.5%, which indicated that it does not exist adaptable problems for strain BSM-30 to the marine environment, being these features consistent with our research purpose.

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