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The Screening of Biopolymer Bacteria in Porous Media

WU Jingchun[a],*; ZHAO Yang[a]; SHI Fang[a]

[a] Laboratory of Enhanced Oil Recovery of Education Ministry, Northeast Petroleum University, Daqing, China.
* Corresponding author.

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Abstract

MEOR has advantages of less polluting environment and low cost, which can be extended to the volume of the biopolymer produced by metabolism, and it is one of the main mechanisms of microbial enhanced oil recovery. The fermentation, the study of biopolymers produced growth and metabolism of bacteria rules; after using the orthogonal experimental method and optimized the optimum culture conditions, through the indoor driving oil experimental evaluation of biological polymer flooding effect. The optimal bio polymer producing strain DJ was screened out. The strain had the ability to produce polymer and the product quantity was high. The effect of the biopolymer flooding was good. DJ strain optimum culture time for 72 hours, the optimal fermentation culture conditions: fermentation temperature 35 degrees Celsius; shaking speed r/min; 10 mL of inoculum; fermentation culture medium formula: glucose 40 g/L, 20 g/L of corn steep liquor, corn starch 2 G/L, $NaNO_3$ 3 g/L, KH_2PO_4 0.5 g/L, $Na_2HPO_4 \cdot 12H_2O$ 1 g/L, $MgSO_4 \cdot 7H_2O$ 0.5 g/L, $CaCl_2$ 0.05 g/L, pH 7.2.

Key words: MEOR; Biopolymer; Viscosity; Screening; Porous media

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INTRODUCTION

Biopolymers are compounds produced by microbial metabolism, which has the role of increasing viscosity[1-2]. Compared with chemical polymer, it has many advantages, such as natural, biodegradable, safe and high activity, which has been widely concerned by many industries all over the world. In the petroleum industry, biopolymers are used to enhance oil recovery, improve the viscosity ratio of oil and water, and so on[3-5]. At present, there are few methods to use for screening biopolymers bacteria[6]. It is mostly used to measure the viscosity of the fermented broth to investigate whether it can produce mucus and the ability of the product[7]. In this paper, the optimal fermentation conditions were determined by orthogonal experiment, the optimal fermentation conditions were determined by the orthogonal experiment.

1. THE FORMULAS OF FERMENTATION MEDIUM

Considering the influence of initial pH, inorganic salt on the experiment, six formulations were developed according to the experience.

Formula 1: Glucose 40 g/L, corn steep liquor 20 g/L, $NaNO_3$ 3 g/L, $Na_2HPO_4 \cdot 12H_2O$ 1 g/L, KH_2PO_4 0.5 g/L, $MgSO_4 \cdot 7H_2O$ 0.5 g/L, $CaCl_2$ 0.05 g/L, pH 7.0.

Formula 2: Glucose 40 g/L, corn steep liquor 20 g/L, $NaNO_3$ 3 g/L, $Na_2HPO_4 \cdot 12H_2O$ 1 g/L, KH_2PO_4 0.5 g/L, $MgSO_4 \cdot 7H_2O$ 0.5 g/L, $CaCl_2$ 0.05 g/L, pH 7.0.

Formula 3: Glucose 40 g/L, corn steep liquor 20 g/L, NaCl 1 g/L, $NaNO_3$ 3 g/L, $Na_2HPO_4 \cdot 12H_2O$ 1 g/L, KH_2PO_4 0.5 g/L, $MgSO_4 \cdot 7H_2O$ 0.5 g/L, $CaCl_2$ 0.05 g/L, pH 7.2.

Formula 4: Glucose 40 g/L, corn steep liquor 20 g/L, corn starch 1 g/L, $NaNO_3$ 3 g/L, $Na_2HPO_4 \cdot 12H_2O$ 1 g/L, KH_2PO_4 0.5 g/L, $MgSO_4 \cdot 7H_2O$ 0.5 g/L, $CaCl_2$ 0.05 g/L, pH 7.2.

Formula 5: Glucose 40 g/L, corn steep liquor 20 g/L, corn starch 2 g/L, $NaNO_3$ 3 g/L, $Na_2HPO_4 \cdot 12H_2O$ 1 g/L, KH_2PO_4 0.5 g/L, $MgSO_4 \cdot 7H_2O$ 0.5 g/L, $CaCl_2$ 0.05 g/L, pH 7.2.

Formula 6: Glucose 40 g/L, corn steep liquor 20 g/L, peptone 1 g/L, $NaNO_3$ 3 g/L, $Na_2HPO_4 \cdot 12H_2O$ 1 g/L, KH_2PO_4 0.5 g/L, $MgSO_4 \cdot 7H_2O$ 0.5 g/L, $CaCl_2$ 0.05 g/L, pH 7.2.

2. THE RESERVOIR COMPATIBILITY OF STRAINS

2.1 The Growth Curves of Strains

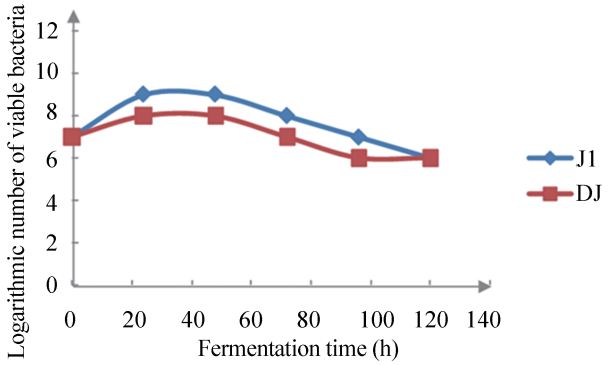

Figure 1
Growth Curves at Reservoir Conditions

Experimental results showed that the strain J1 and DJ grew well in simulated formation environment, the number of bacteria during the culture could be maintained at a higher value. J1 after 24 hours could reach 10^9/mL, and DJ after 24 hours can reach 10^8/mL. Two kinds of bacteria reservoir compatibility were good, J1 was slightly better than DJ. Under reservoir conditions, the growth of each strain curve shown in Figure 1.

2.2 Fermentation Parameters Measurement Results

It was determined that the viscosity of the J1 fermentation broth was 19.37 mPa·s, the viscosity of the DJ fermentation broth was 25.65 mPa·s, DJ ability to produce polymer was higher than J1.

3. THE GROWN METABOLISM RULES OF STRAINS

From Figure 2, Figure 3 showed that the strain J1 and DJ in a fermentation flask growth and metabolism would generally go through three stages: the initial fermentation, the medium fermentation, the last fermentation. During the initial stage of fermentation, the number of bacteria increased, the number of bacteria increased, the product began to accumulate, and the viscosity increased. During the medium fermentation, the number of bacteria remained stable and the product was accumulated. However, with the aging of the cell, the metabolism ability of the strains decreased, which made the viscosity of fermentation liquor increased to a certain value. The third stage is the last fermentation, the aging and death of the cell increased, the number of bacteria was decreased, and the ability of producing polymer was lost. During the whole fermentation process, the pH value increased slowly, but the change was not very large.

According to the change curve of viscosity with fermentation time, the results showed that the viscosity of J1 and DJ in the late fermentation stage was basically constant, so the two strains could be harvested at the end of fermentation. At the same time, considering the production cost, the best harvest time of s J1 was set to 96 hours, and the best harvest time of DJ was 72 hours.

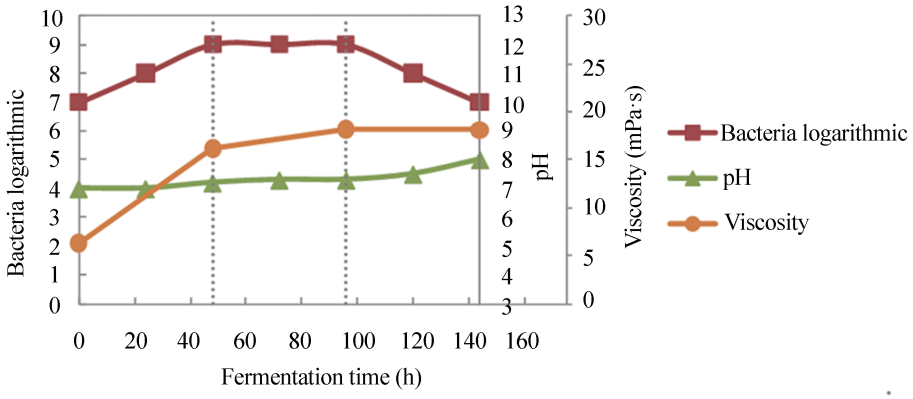

Figure 2
The Fermentation Curve of J1

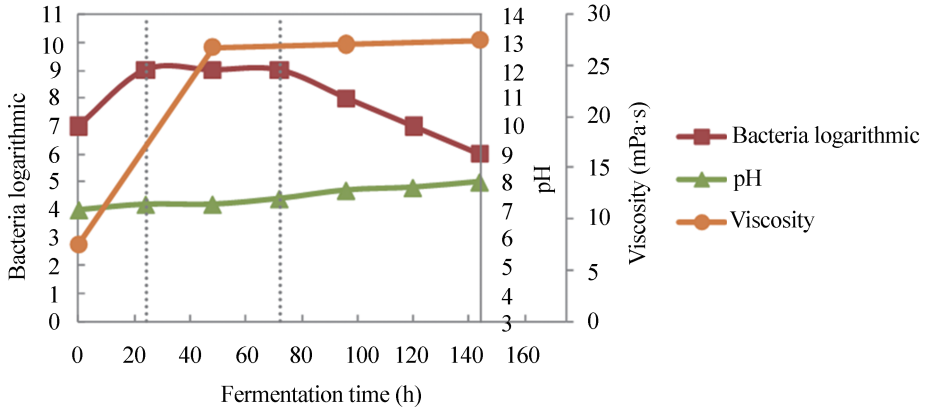

Figure 3
The Fermentation Curve of DJ

4. THE OPTIMAL FERMENTATION CONDITIONS

4.1 The Results of Orthogonal Experiment and Variance Analysis

The results of orthogonal experiment of strain J1 and DJ are shown in Tables 1 and 2.

From Table 1, strain J1 orthogonal experimental results by analysis of variance, $F_A > F_{0.1}$ (5,6), $F_B < F_{0.1}$ (2,6), $F_C < F_{0.1}$ (2,6), $F_D > F_{0.1}$ (2,6). That factor A and D in the significant level of 0.1 significantly, factor B, C was not significant, namely the fermentation flask culture medium formula and shaking speed of J1 biopolymer producing ability has a significant effect, and effect of inoculation and fermentation temperature was not obvious. Sample mean of the contrast factors, the optimal level of each factor was selected as follows: The level of A factor was 5, the level of B was 2, the level of C was 3, and the level of D was 1. Because of A, D were significant factors, must choose the optimal level, namely medium five, shaking speed 80r/min. While C, B was not a significant factor, but in order to shorten the training time, take B factors 2 levels, C factors 3 levels, that is, the inoculation amount of 10 mL, fermentation temperature 35 ℃. Therefore, the optimum fermentation conditions of strain J1: Formulation 5, 10 mL of inoculum, fermentation temperature 35 ℃, rotation speed 80 r/min.

Table 1
Orthogonal Experiment Results and Analysis of J1

Number	A Formula Medium	B Inoculation amount	C Fermentation temperature	D Shaking speed	Viscosity mPa·s
1	1	1	1	1	19.72
2	1	2	2	2	22.36
3	1	3	3	3	17.45
4	2	1	1	2	18.27
5	2	2	2	3	20.63
6	2	3	3	1	23.54
7	3	1	2	1	22.89
8	3	2	3	2	23.4
9	3	3	1	3	18.52
10	4	1	3	3	25.33
11	4	2	1	1	28.55
12	4	3	2	2	27.88
13	5	1	2	3	25.31
14	5	2	3	1	29.77

To be continued

Continued

Number		A Formula Medium	B Inoculation amount	C Fermentation temperature	D Shaking speed	Viscosity mPa·s
15		5	3	1	2	28.60
16		6	1	3	2	22.96
17		6	2	1	3	20.78
18		6	3	2	1	21.15
Sample average	$\overline{X}_1$	19.843	22.413	22.407	24.270	Ensemble average $\overline{X}$ = 23.173
	$\overline{X}_2$	20.813	24.248	23.370	23.912	
	$\overline{X}_3$	21.603	22.857	23.742	21.337	
	$\overline{X}_4$	27.253	——	——	——	
	$\overline{X}_5$	27.893	——	——	——	
	$\overline{X}_6$	21.630	——	——	——	
F ratio		17.877	2.712	1.404	7. 575	$F_{0.1}(5,6) = 9.16$ $F_{0.1}(2,6) = 8.53$

From Table 2, the results variance analysis of strain DJ was the same as strain J1[8], $F_A > F_{0.1}$ (5,6), $F_B < F_{0.1}$ (2,6), $F_C < F_{0.1}$ (2,6), $F_D > F_{0.1}$ (2,6), the fermentation bottle culture medium formula and shaking speed on the viscosity of the fermentation broth have significant effect, inoculation and fermentation temperature effect is not obvious. Sample mean of the contrast factors, the optimal level of each factor was selected as follows: the level of A factor was 5, the level of B was 2, the level of C was 3, and the level of D was 1. In the same way, the level of A selection was 5, the level of B was 2, the level of C was 3, and the level of D was 1. That is the best fermentation condition of strain DJ: Formulation 5, rotation speed 80 r/min, 10 mL inoculation, fermentation temperature 35 ℃.

4.2 The Results of the Optimal Fermentation Conditions

Under the optimum fermentation conditions[9], the viscosity of J1 fermentation broth was 29.82 mPa·s and DJ fermentation broth was 40.67 mPa·s, in a reasonable range, the optimum conditions were in accord with the requirements. It is clear that the ability of DJ to produce biopolymers is higher than J1, so the choice of DJ is used in the composite system.

Table 2
Orthogonal Experiment Results and Analysis of Strain DJ

Number	A Formula medium	B Inoculation amount	C Fermentation temperature	D Shaking speed	Viscosity mPa·s
1	1	1	1	1	30.10
2	1	2	2	2	29.85
3	1	3	3	3	25.47
4	2	1	1	2	28.55
5	2	2	2	3	26.23
6	2	3	3	1	30.92
7	3	1	2	1	27.86
8	3	2	3	2	29.33
9	3	3	1	3	26.97
10	4	1	3	3	32.21
11	4	2	1	1	35.13

To be continued

Continued

Number		A Formula medium	B Inoculation amount	C Fermentation temperature	D Shaking speed	Viscosity mPa·s
12		4	3	2	2	32.89
13		5	1	2	3	33.54
14		5	2	3	1	39.88
15		5	3	1	2	36.26
16		6	1	3	2	31.73
17		6	2	1	3	30.90
18		6	3	2	1	32.15
Sample average	$\overline{X}_1$	28.473	30.665	31.318	32.673	Ensemble average $\overline{X}$ = 31.109
	$\overline{X}_2$	28.567	31.887	30.420	31.435	
	$\overline{X}_3$	28.053	30.777	31.590	29.220	
	$\overline{X}_4$	33.410	——	——	——	
	$\overline{X}_5$	36.560	——	——	——	
	$\overline{X}_6$	31.593	——	——	——	
F ratio		32.581	2.737	2.250	17.197	$F_{0.1}(5,6) = 9.16$ $F_{0.1}(2,6) = 8.53$

CONCLUSION

(a) The compatibility of J1 and DJ is better than that of J1, and the ability of to produce biopolymer is higher than that of DJ, so the polymer of DJ fermentation liquid is used as the composite system.

(b) The growth and metabolism of strain DJ in the fermentation bottle will go through three stages: the initial stage of fermentation, the middle stage of fermentation, the last stage of fermentation, the best harvest time is 72 hours.

(c) The optimal fermentation culture conditions: fermentation temperature 35 degrees Celsius; shaking speed r/min; 10 ml of inoculum; fermentation culture medium formula: glucose 40 g/L, 20 g/L of corn steep liquor, corn starch 2 G/L, $NaNO_3$ 3 g/L, KH_2PO_4 0.5 g/L, $Na_2HPO_4 \cdot 12H_2O$ 1 g/L, $MgSO_4 \cdot 7H_2O$ 0.5 g/L, $CaCl_2$ 0.05 g/L, pH 7.2.

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