·生物磁学·

Culture Conditions Optimization of Magnetotactic Bacteria WM-1

LI Wen-bing, ZHOU Peng-peng, YU Long-jiang

(College of Life Science & Technology, Huazhong University of Science & Technology, Wuhan, 430074, P.R. China)

ABSTRACT: The culture of Magnetospirillum magneticum WM-1 depends on several control factors that have great effect on the magnetic cells concentration. Investigation into the optimal culture conditions needs a large number of experiments. So it is desirable to minimize the number of experiments and maximize the information gained from them. The orthogonal design of experiments and mathematical statistical method are considered as effective methods to optimize the culture condition of magnetotactic bacteria WM-1 for high magnetic cells concentration. The effects of the four factors, such as pH value of medium, oxygen concentration of gas phase in the serum bottle, C:C ($m_{tartaric\ acid}$: $m_{succinic\ acid}$) ratio and $NaNO_3$ concentration, are simultaneously investigated by only sixteen experiments through the orthogonal design L16(4^4) method. The optimal culture condition is obtained. At the optimal culture condition (pH 7.0, a oxygen concentration 4.0%, C: C ($m_{tartaric\ acid}$: $m_{succinic\ acid}$) ratio 1:2 and $NaNO_3$ 100 mg l^{-1}), the magnetic cells concentration is promoted to 6.5×10^7 cells ml^{-1}, approximately 8.3% higher than that under the initial conditions. The pH value of medium is very important factor for magnetic cells concentration. It can be proved that the orthogonal design of experiment is of 90% confidence. The results from the hysteresis of WM-1 shows that H_c = 230 Oe, M_s = 0.9 emu/g dry wt. Cells, and M_r / M_s = 0.50.

Key words: Magnetotsctic bacteria; Orthogonal design; Optimization; Magnetic property; Magnetosomes

1 Introduction

Magnetotactic bacteria have been isolated from fresh and marine sediments and are known to produce intracellularly magnetic particles (magnetosomes, usually made of Fe_3O_4)[1]. Current researches of magnetotactic bacteria focus on two aspects [2]. One illustrates the biomineralization process during synthesis of magnetosomes. The other investigates practical applications of this intriguing magnetic nano-particles. Bacterial magnetic particles are magnetic single-domain particles (50-100 nm in size)[3], which act as compass needles and enable the bacteria to orientate in the geomagnetic field [4]. There is an intriguing research field for the synthesis of biocompatible magnetosomes with stable lipid membrane through magnetotactic bacteria, which can be applied to immunoassay[5], target therapy[6], molecule labeled and magnetic separation[7,8]. These applications emphasize the need to enhance magnetic cells production for biotechnological applications.

The magnetic cells concentration is a very important factor for obtaining the bacterial magnetic particles [9]. The magnetic cells concentration strongly depends on many control factors of culture conditions, such as pH of medium, oxygen concentration, carbon source and nitrogen source[10]. Consequently, the investigation into the optimal culture conditions needs a large number of experiments. So it is desirable to minimize the number of experiments and maximize the amount of information acquired from them. In this paper, the mathematical statistical methods can be used to obtain this goal.

The orthogonal design of experiments and the analysis of variance are mathematical statistical methods[11,12]. In this paper, the culture condition optimization based on the orthogonal design of experiments is used for the fermentation cultivation of magnetotactic bacteria WM-1. In general, we have to do 4 × 4 × 4 × 4 = 256 experiments to investigate the effects of four culture control factors with four levels precisely; however, only sixteen experiments will be sufficient by applying the orthogonal design and the analysis of variance. The hysteresis loop of magnetic cells is also measured.

2 Materials and methods

2.1 Microorgnisms and culture conditions

The Magnetospirillum magneticum WM-1, which has been isolated from the North-lake (114° 31′ E, 30° 36′ N) in Wuhan and stored in our laboratory [13], is maintained in the magnetic spirillum growth medium (MSGM)[14]. The compenents of MSGM are 0.68 g KH_2PO_4, 0.12 g $NaNO_3$, 0.37g tartaric acid, 0.37g succinic acid, and 0.035g ascorbic acid in 1.0 l distilled water. The medium is supplemented with 10.0 ml Wolfe,s vitamin solution, 5.0ml Wolfe, s mineral solution, 2.0 ml ferric quinate solution (0.45 g $FeCl_3 \cdot 6H_2O$ and 0.19g quinic acid in 100 ml distilled water). The initial pH of the culture medium is adjusted to

Author synopsis: Wenbing Li, (1973-), male, doctoral student,
Research direction: Resource Microbiology and Technology.
The author contributed equally to this work with the first author.
Corresponding author. Tel.:+86-27-8779-2264; fax:+86-27-8779-2265.
E-mail: yulongjiang@mail.hust.edu.cn (LJ Yu); liwenbing@126.com (WB Li).
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7.0 ± 0.1 using H_2SO_4 or NaOH. The concentration of oxygen in each 135 ml serum bottle is achieved by the method of replacement[15]. The media are autoclaved at 121℃ for 20 min.

Cells are precultured to late exponential phase (10^7 cells/ml) in a 135 ml serum bottle, and 1 ml transferred as inoculum to 135 ml serum bottle containing 50 ml MSGM. The starting density of cells after inoculation is 2×10^5cells ml^{-1}. The culture is incubated at 28 ℃ for 7 days. All experiments are performed under the same culture condition mentioned above except the serum bottle containing different medium prepared according to the orthogonal experiment design, and conducted in triplicate. Furthermore, ferric quinate solution sterilized by filtration is added to MSGM at a final concentration of 40 μ M (the concentration of ferric quinate is fixed in orthogonal experiment, because the stain WM-1 can produce the maximally magnetic cells concentration at this condition (data not shown)).

2.2 Orthogonal experiment

Orthogonal experiment design is based on the principle of statistical mathematics and the experiments are arranged scientifically to solve multiple-factor optimization requirements with reduced numbers of experiments conducted. The influence of factors, specifically pH value of medium, O_2 concentration (V:V, %) of gas phase in the serum bottle, C:C ($m_{tartaric\ acid}$: $m_{succinic\ acid}$) ratio and $NaNO_3$ concentration (mg l^{-1}), on the cell concentration of magnetotactic bacteria WM-1 is optimized by an orthogonal experiment as shown in Table 1.

Table 1 Factor and levels of the orthogonal experiment

Level	Factor			
	A	B	C	D
	pH	O_2 concentration (%)	C:C	ρ_{NaNO3} (mg l^{-1})
1	6.0	1.0	1:1	100
2	6.5	2.0	1:2	120
3	7.0	3.0	1:3	140
4	7.5	4.0	1:4	160

2.3 Analyses

Magnetic cells concentration is determined by haemocytometry [16]. Tension of oxygen in the gas phase is determined by gas chromatography (GC-14A, Shimadzu, Japan) [2]. The magnetosomes in cells are determined by transmission electron microscope (JEM-1230, JEOL, Japan)[17]. Fig. 1 shows the representative morphology of magnetosomes produced by the WM-1.To measure the hysteresis loop of WM-1 samples, we used a Quantum Designs Magnetic Property MeasurementSystem (MPMS), a low-temperature superconducting quantum interference device (SQUID) magnetometer[18].

3 Results and discussion

3.1 Culture conditions optimization

Table 2 shows the orthogonal design L16 (44) and the magnetic cells concentration results of the experiments. In each experiment, the ferric concentration in the medium is initially 40 μ M, the culture is incubated at 28 ℃ for 7 days. The effects of each level of all factors are shown in Table 2. The optimal culture condition is obtained if the sum of the Number values of a factor at a level is maximum, because the larger the Number, the more the magnetic cells. From the results of Tabl · 2, the primary and secondary order that have effect on the fermentation of magnetotactic bacteria WM-1, is A > B > C > D through range analysis. The optimal culture conditions are at a pH value of 7.0, an oxygen concentration of 4.0%, a NaNO3 of 100 mg l-1, and a C: C ($m_{tartaric\ acid}$: $m_{succinic\ acid}$) ratio of 1:2.

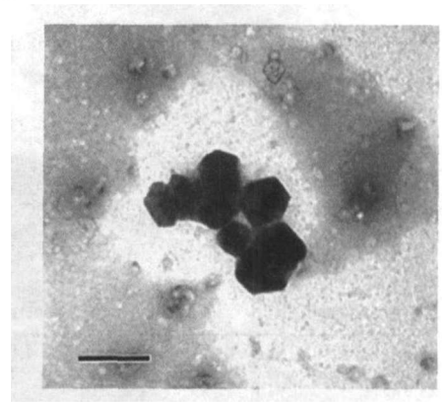

Fig. 1 Transmission electron micrograph of magnetosomes of strain WM-1 placed over a Formvar film. The magnetosomes are arrayed random. Shapes of magnetosomes crystals are mainly cuboidal prisms. The bar represents 100 nm.

Table 2 Orthogonal design L16(4^4) and results of orthogonal experiments

No.	Factors				Magnetic cells number
	A	B	C	D	($\times 10^7$ cells ml^{-1})
1	1	1	1	1	1.5
2	1	2	2	2	1.6
3	1	3	3	3	1.7
4	1	4	4	4	1.5
5	2	1	2	3	2.7
6	2	2	1	4	3.2
7	2	3	4	1	2.9
8	2	4	3	2	4.0
9	3	1	3	4	3.1
10	3	2	4	3	4.5
11	3	3	1	2	5.3
12	3	4	2	1	6.0
13	4	1	4	2	1.8
14	4	2	3	1	1.9
15	4	3	2	4	2.0
16	4	4	1	3	2.3
K1	1.575	2.275	3.075	3.000	
K2	3.200	2.800	3.175	2.475	
K3	4.725	2.975	2.800	3.175	
K4	2.000	3.450	2.450	2.850	
R	3.150	1.175	0.725	0.700	

The analysis of the variance method is used to investigate the effect of each factor and the confidence level of the experiment. The analytical results are shown in Table 3. The symbol Q represents the sums of squares, which can be calculated by the following formula [11,12]:

$$Q=\sum_{i=1}^{n}(y_i-\bar{y})^2=\sum_{i=1}^{n}y_i^2-\frac{1}{n}\left(\sum_{i=1}^{n}y_i\right)^2,$$

Here, n is the number of the experiments, i represents an experiment (i = 1,2,3, ···,n), and $\bar{y}$ is overall mean ($\bar{y}$= 2.88× 10^7 cells ml^{-1}).

Q_A is the sum of squares of the A factor, which can be acquired by Liu et al. [11]and Liu[12]

$$Q_A=\frac{1}{b}\sum_{l=1}^{b}\left(\sum_{m=1}^{a}y_{lm}\right)^2-\frac{1}{n}\left(\sum_{i=1}^{n}y_i\right)^2$$

where ylm is the measured value, which represents a factor at the mth level with the lth experiment (m = 1,2,···,a; l = 1,2,···,b).

Table 3 Square variance analysis for orthogonal design of experiments

Factor	Q	f	M	F	F0.90(3,12)	Effect
A	23.935	3	7.978	3.291	2.610	*
B	2.825	3	0.942	0.388	2.610	
C	1.265	3	0.422	0.174	2.610	
D	1.065	3	0.355	0.146	2.610	
Error	29.09	12	2.424			

The symbol f represents degrees of freedom. The symbol MA represents the mean square of the A factor; thus $M_A = Q_A/f_A$. The symbol F represents a test statistic according to the F distribution; $F_A = M_A/M_A$. On analysis of Table 3, we suppose that the confidence level of experiments is 90%. According to the F statistic distribution table, $F_{0.90}$ (3,12) = 2.61. If $F_A > 2.61$, the effect of the A factor is recognized as an important factor for the experiment. Since the F statistic of the pH value is the largest, the pH value is the important control factor for the cells concentration of Magnetospirillum magneticum WM-1. Furthermore, the other three factors (O_2 concentration, C:C, and $NaNO_3$) are not statistically significant. The results of the orthogonal experiment are considered at the 90% confidence level.

3.2 Test of the optimal culture condition

The optimal culture condition determined with the orthogonal experiment (pH 7.0, a oxygen concentration 4.0%, C: C ($m_{tartaric\ acid}$: $m_{succinic\ acid}$) ratio 1:2 and $NaNO_3$ 100 mg l^{-1}), is used to incubate the Magnetospirillum magneticum WM-1. Table 4 shows the results of the verified experiments conducted in triplicate. From Table 4, the mean cells number is 6.5× 10^7 cells ml^{-1}, which is greater than 6.0× 10^7 cells ml^{-1}, approximately 8.3% higher than that under the initial conditions. The results of the verified experiments are obviously better than the result of the orthogonal experiment.

3.3 Hysteresis measurement

Measurement of the freeze-dried whole cells at 300 K is made with a MPMS SQIUD magnetometer. Fig. 2 shows the curve of the WM-1. H_c = 230 Oe, M_s = 0.9 emu/g dry wt. Cells, and M_r / M_s = 0.50. The remanence ratio is consistent with the theoretical value of 0.5 for a randomly oriented ensemble of uniaxial SD particles, and agrees with an earlier study by Moskowitz et al.[19].

4 Conclusion

Table 4 The results of repeated experiments at optimal culture conditions

No.	Initial number (× 10^5 cells ml^{-1})	Final number (× 10^7 cells ml^{-1})
1	2	6.4
2	2	6.5
3	2	6.5
Mean	2	6.5

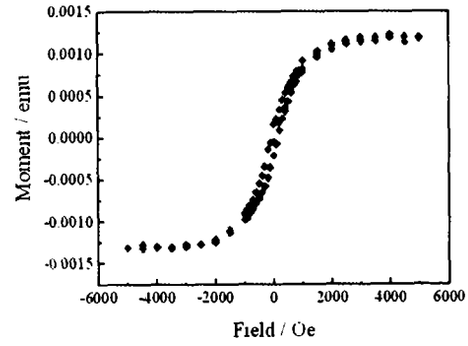

Fig. 2 Hysteresis loop of freeze-dried whole cells of magnetotactic bacteria WM-1 and measured in SQUID magnetometer.

In this paper, the pH of culture medium directly influences the magnetic cells concentration. The investigation into the optimal culture conditions needs a large number of experiments. Consequently, it is desirable to minimize the number of experiments and maximize the information gained from them. The orthogonal design of experiments and the analysis of variance are considered effective methods to optimize the culture conditions of Magnetospirillum magneticum WM-1 for higher concentration of magnetic cells. Using the orthogonal design of experiments, the effects of four factors, such as pH of medium, oxygen concentration, C:C and $NaNO_3$, are simultaneously investigated by only sixteen experiments. The optimal conditions of culture are at a pH value of 7.0, an oxygen concentration of 4.0%, a C: C ratio of 1:2 and a $NaNO_3$ of 100 mg l^{-1}. At the optimal condition, magnetic cells concentration is promoted to 6.5× 10^7 cells ml^{-1}. The pH value of medium is very important factor for magnetic cells concentration. It can be proved that the results of the orthogonal experiment are at the 90% confidence level. Hc value of freeze-dried whole WM-1 cells is 230 Oe, Ms = 0.9 emu/g dry wt. Cells, and M_r / M_s = 0.50.

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趋磁细菌 WM-1 的培养条件优化研究

李文兵 周蓬蓬 余龙江

(华中科技大学 生命科学与技术学院 湖北 武汉 430074)

摘要:采用正交设计实验法研究了趋磁细菌 WM-1 产磁性细胞的培养条件。并利用 L16(44)方案,用 16 个实验完成了 4 种培养条件、4 个水平的优化工作。研究结果表明,培养基的 pH 值是影响趋磁细菌 WM-1 产磁性细胞的重要因素,正交实验结果在 90%的置信区间可信。在最优的培养条件下,即 pH 为 7.0,氧气的浓度为 4%,m 酒石酸:m 琥珀酸为 1:1,$NaNO_3$ 100 mg l^{-1} 条件下,磁性细胞的浓度提高到 6.5×10^7 cells ml^{-1}, 比优化前提高了约 8.3%. 趋磁细菌 WM-1 磁滞回线的测量表明, H_c= 230 Oe, M_s = 0.9 emu/g dry wt. Cells, 及 M_r / M_s = 0.50。

关键词:趋磁细菌; 正交设计; 优化; 磁性; 磁小体

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作者简介:李文兵,(1973-),男,博士研究生,

主要研究方向:资源微生物与技术。

周蓬蓬、等同第一作者,与第一作者有相同的贡献。

通讯作者:余龙江,电话: 027-87792264,

传真: 027-87792265; E-mail: yulongjiang@mail.hust.edu.cn

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